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<b>(54) Title:</b> RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS, COMPOSITIONS AND METHODS OF USE			
<b>(57) Abstract</b> <p>The present invention relates to methods for producing recombinant heterodimeric BMP proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to the recombinant heterodimers and compositions containing them.</p>			
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RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS,  
COMPOSITIONS AND METHODS OF USE

Field of the Invention

5           The present invention relates to a series of novel recombinant heterodimeric proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to methods for obtaining these heterodimers, methods for  
10          producing them by recombinant genetic engineering techniques, and compositions containing them.

Background of the Invention

In recent years, protein factors which are characterized by bone or cartilage growth inducing properties have been isolated and identified. See, e.g., U. S. Patent No. 5,013,649, PCT published application WO90/11366; PCT published application WO91/05802 and the variety of references cited therein. See, also, PCT/US90/05903 which discloses a protein sequence termed OP-1, which is substantially similar to human BMP-7, and has been reported to have osteogenic activity.

A family of individual bone morphogenetic proteins (BMPs), termed BMP-2 through BMP-9 have been isolated and identified. Incorporated by reference for  
25          the purposes of providing disclosure of these proteins

and methods of producing them are co-owned, co-pending U.S. Patent Application SN 721,847 and the related applications recited in its preamble. Of particular interest, are the proteins termed BMP-2 and BMP-4, disclosed in the above-referenced application; BMP-7, disclosed in SN 438,919; BMP-5, disclosed in SN 370,547 and SN 356,033; and BMP-6, disclosed in SN 370,544 and SN 347,559; and BMP-8, disclosed in SN 525,357. Additional members of the BMP family include BMP-1, disclosed in SN 655,578; BMP-9, disclosed in SN 720,590; and BMP-3, disclosed in SN 179,197 and PCT publication 89/01464. These applications are incorporated herein by reference for disclosure of these BMPs.

There remains a need in the art for other 15 proteins and compositions useful in the fields of bone and wound healing.

#### Summary of the Invention

In one aspect, the invention provides a method for producing a recombinant heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof and a 20 polynucleotide sequence encoding a second selected BMP or fragment thereof. The resulting co-expressed, 25 biologically active heterodimer is isolated from the culture medium.

According to one embodiment of this invention,

the host cell may be co-transfected with one or more vectors containing coding sequences for one or more BMPs. Each BMP polynucleotide sequence may be present on the same vector or on individual vectors transfected into the cell. Alternatively, the BMPs or their fragments may be incorporated into a chromosome of the host cell. Additionally, a single transcription unit may encode single copy of two genes encoding a different BMP.

According to another embodiment of this invention, the selected host cell containing the two polypeptide encoding sequences is a hybrid cell line obtained by fusing two selected, stable host cells, each host cell transfected with, and capable of stably expressing, a polynucleotide sequence encoding a selected first or second BMP or fragment thereof.

In another aspect of the present invention, therefore, there are provided recombinant heterodimeric proteins comprising a protein or fragment of a first BMP in association with a protein or fragment of a second BMP. The heterodimer may be characterized by bone stimulating activity. The heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8; or a protein or fragment of BMP-4 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8. In further embodiments the heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or

fragment of either BMP-1, BMP-3 or BMP-4. BMP-4 may also form a heterodimer in association with BMP-1, BMP-2 or a fragment thereof. Still further embodiments may comprise heterodimers involving combinations of BMP-5, BMP-6, BMP-7 and BMP-8. For example, the heterodimers may comprise 5 BMP-5 associated with BMP-6, BMP-7 or BMP-8; BMP-6 associated with BMP-7 or BMP-8; or BMP-7 associated with BMP-8. These heterodimers may be produced by co-expressing each protein in a selected host cell and isolating the heterodimer from the culture medium.

10 As a further aspect of this invention a cell line is provided which comprises a first polynucleotide sequence encoding a first BMP or fragment thereof and a second polynucleotide sequence encoding a second BMP or fragment thereof, the sequences being under control of 15 one or more suitable expression regulatory systems capable of co-expressing the BMPs as a heterodimer. The cell line may be transfected with one or more than one polynucleotide molecule. Alternatively, the cell line 20 may be a hybrid cell line created by cell fusion as described above.

Another aspect of the invention is a 25 polynucleotide molecule or plasmid vector comprising a polynucleotide sequence encoding a first selected BMP or fragment thereof and a polynucleotide sequence encoding a second selected BMP or fragment thereof. The sequences are under the control of at least one suitable regulatory

sequence capable of directing co-expression of each protein or fragment. The molecule may contain a single transcription unit containing a copy of both genes, or more than one transcription unit, each containing a copy 5 of a single gene.

As still another aspect of this invention there is provided a method for producing a recombinant dimeric or heterodimeric protein having bone stimulating activity in a prokaryotic cell comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof; culturing a second selected host cell containing a polynucleotide sequence encoding a second selected BMP or fragment thereof; isolating monomeric forms of each BMP protein 10 from the culture medium and co-assembling a monomer of the first protein with a monomer of the second protein. The first protein and the second protein may be the same or different BMPs. The resulting biologically active 15 dimer or heterodimer is thereafter isolated from the mixture. Preferred cells are E. coli.

Thus, as further aspects of this invention recombinant BMP dimers or heterodimers produced in eukaryotic cells are provided, as well as suitable vectors or plasmids, and selected transformed cells 20 useful in such a production method.

Other aspects and advantages of the present invention are described further in the following detailed

description of preferred embodiments of the present invention.

Brief Description of the Figures

Figure 1 provides the DNA and amino acid sequences of human BMP-2 (SEQ ID NOS: 1 and 2).  
5

Figure 2 provides the DNA and amino acid sequences of human BMP-4 (SEQ ID NOS: 3 and 4).

Figure 3 provides the DNA and amino acid sequences of human BMP-7 (SEQ ID NOS: 5 and 6).

10 Figure 4 provides the DNA and amino acid sequences of human BMP-6 (SEQ ID NOS: 7 and 8).

Figure 5 provides the DNA and amino acid sequences of human BMP-5 (SEQ ID NOS: 9 and 10).

15 Figure 6 provides the DNA and amino acid sequences of human BMP-8 (SEQ ID NOS: 11 and 12).

Figure 7 provides the DNA sequence of vector pALB2-781 containing the mature portion of the BMP-2 gene (SEQ ID NOS: 13 and 14).

20 Figure 8 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the W20 alkaline phosphatase assay.

Figure 9 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the BGP (osteocalcin) assay.

25 Figure 10 provides a comparison of the W-20 activity of E. coli produced BMP-2 and BMP-2/7 heterodimer.

Figure 11 depicts BMP-3 DNA and amino acid sequence.

Figure 12 provides a comparison of BMP-2 and BMP-2/6

in the W-20 assay.

Figure 13 provides a comparison of the in vivo activity of BMP-2/6 and BMP-2.

Figure 14 provides a comparison of BMP-2, BMP-6 and  
5 BMP-2/6 in vivo activity.

#### Detailed Description of the Invention

The present invention provides a method for producing recombinant heterodimeric proteins having bone stimulating activity, as well as the recombinant 10 heterodimers themselves, and compositions containing them for bone-stimulating or repairing therapeutic use.

As used throughout this document, the term 'heterodimer' is defined as a biologically-active protein construct comprising the association of two different BMP 15 protein monomers or active fragments thereof joined through at least one covalent, disulfide linkage. A heterodimer of this invention may be characterized by the presence of between one to seven disulfide linkages between the two BMP component strands.

According to the present invention, therefore, 20 a method for producing a recombinant BMP heterodimer according to this invention comprises culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or a biologically active 25 fragment thereof and a polynucleotide sequence encoding a second selected BMP or a fragment thereof. The resulting

co-expressed, biologically active heterodimer is formed within the host cell, secreted therefrom and isolated from the culture medium. Preferred embodiments of methods for producing the heterodimeric proteins of this invention, are described in detail below and in the following examples. Preferred methods of the invention involve known recombinant genetic engineering techniques [See, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual:", 2d edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)]. However, other methods, such as conventional chemical synthesis may also be useful in preparing a heterodimer of this invention.

BMP heterodimers generated by this method are produced in a mixture of homodimers and heterodimers. This mixture of heterodimers and homodimers may be separated from contaminants in the culture medium by resort to essentially conventional methods, such as classical protein biochemistry or affinity antibody columns specific for one of the BMPs making up the heterodimer. Additionally, if desired, the heterodimers may be separated from homodimers in the mixture. Such separation techniques allow unambiguous determination of the activity of the heterodimeric species. Example 4 provides one presently employed purification scheme for this purpose.

Preferably the recombinant heterodimers of this

invention produced by these methods involve the BMPs designated human BMP-2, human BMP-4, human BMP-5, human BMP-6, human BMP-7 and BMP-8. However, BMP-3 has also been determined to form an active heterodimer with BMP-2.

5 Other species of these BMPs as well as BMPs than those specifically identified above may also be employed in heterodimers useful for veterinary, diagnostic or research use. However, the human proteins, specifically those proteins identified below, are preferred for human

10 pharmaceutical uses.

Human BMP-2 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 1. Human BMP-2 proteins are further characterized as disulfide-linked dimers and homodimers of mature BMP-2 subunits. Recombinantly-expressed BMP-2 subunits include protein species having heterogeneous amino termini. One BMP-2 subunit is characterized by comprising amino acid #249 (Ser) - #396 (Arg) of Figure 1 (SEQ ID NOS: 1 and 2). Another BMP-2 subunit is characterized by comprising amino acid #266 (Thr) - #396 (Arg) of Figure 1. Another BMP-2 subunit is characterized by comprising amino acid #296 (Cys) - #396 (Arg) of Figure 1. A mature BMP-2 subunit is characterized by comprising amino acid #283 (Gln) - #396 (Arg) of Figure 1. This latter subunit is the presently most abundant protein species which results from recombinant expression of BMP-2 (Figure 1).

However, the proportions of certain species of BMP-2 produced may be altered by manipulating the culture conditions. BMP-2 may also include modifications of the sequences of Figure 1, e.g., deletion of amino acids #241-280 and changing amino acid #245 Arg to Ile, among other changes.

As described in detail in United States Patent Application SN 721,847, incorporated by reference herein, human BMP-2 may be produced by culturing a cell 10 transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #356 to #1543 in Figure 1 and recovering and purifying from the culture medium one or more of the above-identified protein species, substantially free from other proteinaceous materials 15 with which it is co-produced. Human BMP-2 proteins are characterized by the ability to induce bone formation. Human BMP-2 also has in vitro activity in the W20 bioassay. Human BMP-2 is further characterized by the ability to induce cartilage formation. Human BMP-2 may 20 be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-4 is characterized by containing 25 substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 2 (SEQ ID NOS: 3 and 4). Human BMP-4 proteins are

further characterized as disulfide-linked dimers and homodimers of mature BMP-4 subunits. Recombinantly-expressed BMP-4 subunits may include protein species having heterogeneous amino termini. A mature subunit of 5 human BMP-4 is characterized by an amino acid sequence comprising amino acids #293 (Ser) - #408 (Arg) of Figure 2. Other amino termini of BMP-4 may be selected from the sequence of Figure 2. Modified versions of BMP-4, including proteins further truncated at the amino or 10 carboxy termini, may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 721,847, BMP-4 may be produced by culturing a cell transformed with a DNA sequence 15 comprising the nucleotide coding sequence from nucleotide #403 to nucleotide #1626 in Figure 2 and recovering and purifying from the culture medium a protein containing the amino acid sequence from amino acid #293 to #408 as shown in Figure 2, substantially free from other 20 proteinaceous materials with which it is co-produced. BMP-4 proteins are capable of inducing the formation of bone. BMP-4 proteins are capable of inducing formation of cartilage. BMP-4 proteins are further characterized by the ability to demonstrate cartilage and/or bone 25 formation activity in the rat bone formation assay.

Human BMP-7 is characterized by containing substantially the entire sequence, or fragments, of the

amino acid sequence and DNA sequence disclosed in Figure 3. Human BMP-7 proteins are further characterized as disulfide-linked dimers and homodimers of mature BMP-7 subunits. Recombinantly-expressed BMP-7 subunits include 5 protein species having heterogeneous amino termini. One BMP-7 subunit is characterized by comprising amino acid #293 (Ser) - #431 (His) of Figure 3 (SEQ ID NOs: 5 and 6). This subunit is the most abundantly formed protein produced by recombinant expression of the BMP-7 sequence. 10 Another BMP-7 subunit is characterized by comprising amino acids #300 (Ser) - #431 (His) of Figure 3. Still another BMP-7 subunit is characterized by comprising amino acids #316 (Ala) - #431 (His) of Figure 3. Other amino termini of BMP-7 may be selected from the sequence 15 of Figure 3. Similarly, modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-7 may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 438,919, BMP-7 may be produced by 20 culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #97 to nucleotide #1389 in Figure 3 and recovering and purifying from the culture medium a protein containing 25 the amino acid sequence from amino acid #293 to #431 as shown in Figure 3, substantially free from other proteinaceous or contaminating materials with which it is

co-produced. These proteins are capable of stimulating, promoting, or otherwise inducing cartilage and/or bone formation.

Human BMP-6 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 4. Human BMP-6 proteins are further characterized as disulfide-linked dimers of mature BMP-6 subunits. Recombinantly-expressed BMP-6 subunits may include protein species having heterogeneous amino termini. One BMP-6 subunit is characterized by comprising amino acid #375 (Ser) - #513 (His) of Figure 4 (SEQ ID NOS: 7 and 8). Other amino termini of BMP-6 may be selected from the sequence of Figure 4. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-6 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 490,033, incorporated by reference herein, human BMP-6 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #160 to #1698 in Figure 4 and recovering and purifying from the culture medium a protein comprising amino acid #375 to #513 of Figure 4, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced. Human BMP-6 may be further characterized by

the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Human BMP-5 is characterized by containing substantially the entire sequence, or fragments, of the 5 amino acid sequence and DNA sequence disclosed in Figure 5 (SEQ ID NOS: 9 and 10). Human BMP-5 proteins are further characterized as disulfide-linked dimers of mature BMP-5 subunits. Recombinantly-expressed BMP-5 subunits may include protein species having heterogeneous 10 amino termini. One BMP-5 subunit is characterized by comprising amino acid #329 (Ser) - #454 (His) of Figure 5. Other amino termini of BMP-5 may be selected from the sequence of Figure 5. Modified versions, including 15 proteins further truncated at the amino or carboxy termini, of BMP-5 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 588,227, incorporated by reference herein, human BMP-5 may be produced by culturing a cell 20 transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #701 to #2060 in Figure 5 and recovering and purifying from the culture medium a protein comprising amino acid #329 to #454 of Figure 5, substantially free from other proteinaceous materials or 25 other contaminating materials with which it is co-produced. Human BMP-5 may be further characterized by the ability to demonstrate cartilage and/or bone

formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-8 is characterized by containing substantially the entire sequence, or fragments, of the 5 amino acid sequence and DNA sequence disclosed in Figure 6. Human BMP-8 proteins may be further characterized as disulfide-linked dimers of mature BMP-8 subunits. Recombinantly-expressed BMP-8 subunits may include 10 protein species having heterogeneous amino termini. A BMP-8 sequence or subunit sequence comprises amino acid #143 (Ala) - #281 (His) of Figure 6 (SEQ ID NOS: 11 and 12). Other amino termini of BMP-8 may be selected from the sequence of Figure 6. Modified versions, including 15 proteins further truncated at the amino or carboxy termini, of BMP-8 may also be constructed by resort to conventional mutagenic techniques.

As described generally in United States Patent Application SN 525,357, incorporated by reference herein, and as further described herein, human BMP-8 may be 20 produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #1 to #850 in Figure 6 and recovering and purifying from the culture medium a protein comprising amino acid #143 to #281 of Figure 6, or similar amino 25 acid sequences with heterogenous N-termini, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced.

This BMP-8 may also be produced in E. coli by inserting into a vector the sequence encoding amino acid #143 to 281 of Figure 6 with a Met inserted before amino acid #143. Human BMP-8 may be further characterized by the 5 ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Each above described BMP protein in its native, non-reduced dimeric form may be further characterized by an apparent molecular weight on a 12% Laemmli gel ranging 10 between approximately 28kD to approximately 40kD. Analogs or modified versions of the DNA and amino acid sequences described herein which provide proteins or active fragments displaying bone stimulating or repairing activity in the rat bone formation assay described below 15 in Example 9, are also classified as suitable BMPs for use in this invention, further provided that the proteins or fragments contain one or more Cys residues for participation in disulfide linkages. Useful modifications of these sequences may be made by one of skill in the art with resort to known recombinant genetic engineering techniques. Production of these BMP 20 sequences in mammalian cells produces homodimers, generally mixtures of homodimers having heterologous N termini. Production of these BMP sequences in E.coli 25 produces monomeric protein species.

Thus, according to this invention one recombinant heterodimer of the present invention

comprises the association of a human BMP-2, including,  
e.g., a monomeric strand from a mature BMP-2 subunit as  
described above or an active fragment thereof, bound  
through one or up to seven covalent, disulfide linkages  
5 to a human BMP-5 including, e.g., a monomeric strand from  
a mature BMP-5 subunit as described above or an active  
fragment thereof. Another recombinant heterodimer of the  
present invention comprises the association of a human  
BMP-2, as described above, bound through one or up to  
10 seven covalent, disulfide linkages to a human BMP-6,  
including, e.g., a monomeric strand from a BMP-6 subunit  
as described above or an active fragment thereof.  
Another recombinant heterodimer of the present invention  
comprises the association of a human BMP-2, as described  
15 above, bound through one or up to seven covalent,  
disulfide linkages to a human BMP-7, including, e.g., a  
monomeric strand of a BMP-7 subunit as described above or  
an active fragment thereof. Another recombinant  
heterodimer of the present invention comprises the  
20 association of a human BMP-2, as described above, bound  
through one or up to seven covalent, disulfide linkages  
to a human BMP-8, including, e.g., a monomeric strand of  
a BMP-8 subunit as described above or an active fragment  
thereof.  
25 Still another recombinant heterodimer of the  
present invention comprises the association of a human  
BMP-4, including, e.g., a monomeric strand of a BMP-4

subunit as described above or an active fragment thereof,  
bound through one or up to seven covalent, disulfide  
linkages to a human BMP-5, as described above. Another  
recombinant heterodimer of the present invention  
5 comprises the association of a human BMP-4, as described  
above, bound through one or more covalent, disulfide  
linkages to a human BMP-6, as described above. Another  
recombinant heterodimer of the present invention  
comprises the association of a human BMP-4, as described  
10 above bound through one or more covalent, disulfide  
linkages to a human BMP-7, as described above. Another  
recombinant heterodimer of the present invention  
comprises the association of a human BMP-4, as described  
above, bound through one or more covalent, disulfide  
linkages to a human BMP-8, as described above.  
15

A further recombinant heterodimer of the  
present invention comprises the association of a human  
BMP-2, including, e.g., a monomeric strand from a mature  
BMP-2 subunit as described above or an active fragment  
20 thereof, bound through at least one disulfide linkage to  
a human BMP-3 including, e.g., a monomeric strand from a  
mature BMP-3 subunit as described above or an active  
fragment thereof. Another recombinant heterodimer of the  
present invention comprises the association of a human  
BMP-2, as described above, bound through at least one  
25 disulfide linkage to a human BMP-4, including, e.g., a  
monomeric strand from a BMP-4 subunit as described above

or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-7, including, e.g., a monomeric strand of a BMP-7 subunit as described above or an active fragment thereof. In addition, human BMP-5 may be associated with human BMP-8 bound through at least one disulfide linkage to a human BMP-8 subunit or active fragment thereof.

Still another recombinant heterodimer of the present invention comprises the association of a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof, bound through at least one disulfide linkage to a human BMP-7, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-6, as described above, bound through one or more covalent, disulfide linkages to a human BMP-8, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-7, as described above bound through one or more covalent, disulfide linkages to a

human BMP-8, as described above.

The disulfide linkages formed between the monomeric strands of the BMPs may occur between one Cys on each strand. Disulfide linkages may form between two Cys on each BMP. Disulfide linkages may form between three Cys on each BMP. Disulfide linkages may form between four Cys on each BMP. Disulfide linkages may form between five Cys on each BMP. Disulfide linkages may form between six Cys on each BMP. Disulfide linkages may form between seven Cys on each BMP. These disulfide linkages may form between adjacent Cys on each BMP or between only selected Cys interspersed within the respective protein sequence. Various heterodimers having the same BMP component strands may form with different numbers of disulfide linkages. Various heterodimers having the same BMP component strands may form with disulfide bonds at different Cys locations. Different heterodimers encompassed by this invention having the same BMP components may differ based upon their recombinant production in mammalian cells, bacterial cells, insect or yeast cells.

These recombinant heterodimers may be characterized by increased alkaline phosphatase activity in the W20 mouse stromal cell line bioassay (Example 8) compared to the individual BMP homodimers, one strand of which forms each heterodimer. Further, these heterodimers are characterized by greater activity in the

W20 bioassay than is provided by simple mixtures of the individual BMP dimers. Preliminary characterization of heterodimers measured on the W20 bioassay have demonstrated that heterodimers of BMP-2 with BMP-5, BMP-6 or BMP-7 are very active. Similarly, heterodimers of BMP-4 with BMP-5, BMP-6 or BMP-7 are strongly active in the W20 bioassay.

Heterodimers of this invention may also be characterized by activity in bone growth and stimulation assays. For example, a heterodimer of this invention is also active in the rat bone formation assay described below in Example 9. The heterodimers are also active in the osteocalcin bioassay described in Example 8. Other characteristics of a heterodimer of this invention include co-precipitation with anti-BMP antibodies to the two different constituent BMPs, as well as characteristic results on Western blots, high pressure liquid chromatography (HPLC) and on two-dimensional gels, with and without reducing conditions.

One embodiment of the method of the present invention for producing recombinant BMP heterodimers involves culturing a suitable cell line, which has been co-transfected with a DNA sequence coding for expression of a first BMP or fragment thereof and a DNA sequence coding for expression of a second BMP or fragment thereof, under the control of known regulatory sequences. The transformed host cells are cultured and the

heterodimeric protein recovered and purified from the culture medium.

In another embodiment of this method which is the presently preferred method of expression of the heterodimers of this invention, a single host cell, e.g., a CHO DUKX cell, is co-transfected with a first DNA molecule containing a DNA sequence encoding one BMP and a second DNA molecule containing a DNA sequence encoding a second selected BMP. One or both plasmids contain a selectable marker that can be used to establish stable cell lines expressing the BMPs. These separate plasmids containing distinct BMP genes on separate transcription units are mixed and transfected into the CHO cells using conventional protocols. A ratio of plasmids that gives maximal expression of activity in the W20 assay, generally, 1:1, is determined.

For example, as described in detail in Example 3, equal ratios of a plasmid containing the first BMP and a dihydrofolate reductase (DHFR) marker gene and another plasmid containing a second BMP and a DHFR marker gene can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection, electroporation, microinjection, protoplast fusion or lipofection. Individual DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum by conventional means. DHFR+ cells containing increased gene copies can be selected

for propagation in increasing concentrations of methotrexate (MTX) (e.g. sequential steps in 0.02, 0.1, 0.5 and 2.0 uM MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982); and 5 Kaufman et al, Mol. Cell Biol., 5:1750 (1983). Expression of the heterodimer or at least one BMP linked to DHFR should increase with increasing levels of MTX resistance. Cells that stably express either or both BMP/DHFR genes will survive. However at a high 10 frequency, cell lines stably incorporate and express both plasmids that were present during the initial transfection. The conditioned medium is thereafter harvested and the heterodimer isolated by conventional methods and assayed for activity. This approach can be 15 employed with DHFR-deficient cells.

As an alternative embodiment of this method, a DNA molecule containing one selected BMP gene may be transfected into a stable cell line which already expresses another selected BMP gene. For example as 20 described in detail in Example 3 below, a stable CHO cell line expressing BMP-7 with the DHFR marker (designated 7MB9) [Genetics Institute, Inc] is transfected with a plasmid containing BMP-2 and a second selectable marker gene, e.g., neomycin resistance (Neo). After 25 transfection, the cell is cultured and suitable cells selected by treatment with MTX and the antibiotic, G-418. Surviving cells are then screened for the expression of

the heterodimer. This expression system has the advantage of permitting a single step selection.

Alternative dual selection strategies using different cell lines or different markers can also be used. For example, the use of an adenosine deaminase (ADA) marker to amplify the second BMP gene in a stable CHO cell line expressing a different BMP with the DHFR marker may be preferable, since the level of expression can be increased using deoxycoformycin (DCF)-mediated gene amplification. (See the ADA containing plasmid described in Example 1). Alternatively, any BMP cell line made by first using this marker can then be the recipient of a second BMP expression vector containing a distinct marker and selected for dual resistance and BMP coexpression.

Still another embodiment of a method of expressing the heterodimers of this invention includes transfecting the host cell with a single DNA molecule encoding multiple genes for expression either on a single transcription unit or on separate transcription units. Multicistronic expression involves multiple polypeptides encoded within a single transcript, which can be efficiently translated from vectors utilizing a leader sequence, e.g., from the EMC virus, from poliovirus, or from other conventional sources of leader sequences. Two BMP genes and a selectable marker can be expressed within a single transcription unit. For example, vectors

containing the configuration BMPx-EMC-BMPy-DHFR or BMPx-  
EMC-BMPy-EMC-DHFR can be transfected into CHO cells and  
selected and amplified using the DHFR marker. A plasmid  
may be constructed which contains DNA sequences encoding  
5 two different BMPs, one or more marker genes and a  
suitable leader or regulatory sequence on a single  
transcription unit.

Similarly, host cells may be transfected with a  
single plasmid which contains separate transcription  
10 units for each BMP. A selectable marker, e.g., DHFR, can  
be contained on another transcription unit, or  
alternatively as the second cistron on one or both of the  
BMP genes. These plasmids may be transfected into a  
selected host cell for expression of the heterodimer, and  
15 the heterodimer isolated from the cells or culture medium  
as described above.

Another embodiment of this expression method  
involves cell fusion. Two stable cell lines which  
express selected BMPs, such as a cell line expressing  
20 BMP-2 (e.g., 2EG5) and a cell line expressing BMP-7  
(e.g., 7MB9), developed using the DHFR/MTX gene  
amplification system and expressing BMP at high levels,  
as described in Example 1 and in the above incorporated  
U.S. applications, can be transfected with one of several  
25 dominant marker genes (e.g., neo', hygromycin', GPT).  
After sufficient time in coculture (approximately one  
day) one resultant cell line expressing one BMP and a

dominant marker can be fused with a cell line expressing a different BMP and preferably a different marker using a fusogenic reagent, such as polyethylene glycol, Sendai virus or other known agent.

5           The resulting cell hybrids expressing both dominant markers and DHFR can be selected using the appropriate culture conditions, and screened for coexpression of the BMPs or their fragments. The selected hybrid cell contains sequences encoding both  
10          selected BMPs, and the heterodimer is formed in the cell and then secreted. The heterodimer is obtained from the conditioned medium and isolated and purified therefrom by conventional methods (see e.g., Example 4). The resulting heterodimer may be characterized by methods  
15          described herein.

Cell lines generated from the approaches described above can be used to produce co-expressed, heterodimeric BMP polypeptides. The heterodimeric proteins are isolated from the cell medium in a form substantially free from other proteins with which they are co-produced as well as from other contaminants found in the host cells by conventional purification techniques. The presently preferred method of production is co-transfection of different vectors into CHO cells and methotrexate-mediated gene amplification. Stable cell lines may be used to generate conditioned media containing recombinant BMP that can be purified and

assayed for in vitro and in vivo activities. For example, the resulting heterodimer-producing cell lines obtained by any of the methods described herein may be screened for activity by the assays described in Examples 8 and 9, RNA expression, and protein expression by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The above-described methods of co-expression of the heterodimers of this invention utilize suitable host cells or cell lines. Suitable cell preferably include mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U. S. Patent 4,419,446. Other suitable mammalian cell lines are the CV-1 cell line, BHK cell lines and the 293 cell line. The monkey COS-1 cell line is presently believed to be inefficient in BMP heterodimer production.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention, e.g., Saccharomyces cerevisiae. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g.,

Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another method for producing a biologically active heterodimeric protein of this invention may be employed where the host cells are microbial, preferably bacterial cells, in particular E. coli. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

This method, which may be employed to produce monomers and dimers (both homodimers and heterodimers) is described in European Patent Application No. 433,225, incorporated herein by reference. Briefly, this process involves culturing a microbial host comprising a nucleotide sequence encoding the desired BMP protein linked in the proper reading frame to an expression control sequence which permits expression of the protein and recovering the monomeric, soluble protein. Where the protein is insoluble in the host cells, the water-insoluble protein fraction is isolated from the host cells and the protein is solubilized. After chromatographic purification, the solubilized protein is subjected to selected conditions to obtain the biologically active dimeric configuration of the protein. This process, which may be employed to produce the heterodimers of this invention, is described specifically

in Example 7, for the production of a BMP-2 homodimer.

Another aspect of the present invention provides DNA molecules or plasmid vectors for use in expression of these recombinant heterodimers. These plasmid vectors may be constructed by resort to known methods and available components known to those of skill in the art. In general, to generate a vector useful in the methods of this invention, the DNA encoding the desired BMP protein is transferred into one or more appropriate expression vectors suitable for the selected host cell.

It is presently contemplated that any expression vector suitable for efficient expression in mammalian cells may be employed to produce the recombinant heterodimers of this invention in mammalian host cells. Preferably the vectors contain the selected BMP DNA sequences described above and in the Figures, which encode selected BMP components of the heterodimer. Alternatively, vectors incorporating modified sequences as described in the above-referenced patent applications are also embodiments of the present invention and useful in the production of the vectors.

In addition to the specific vectors described in Example 1, one skilled in the art can construct mammalian expression vectors by employing the sequence of Figures 1-6 or other DNA sequences containing the coding sequences of Figures 1-6 (SEQ ID NOS: 1, 3, 5, 7, 9 and

11), or other modified sequences and known vectors, such  
as pCD [Okayama et al, Mol. Cell Biol., 2:161-170 (1982)]  
and pJL3, pJL4 [Gough et al, EMBO J., 4:645-653 (1985)].  
The BMP DNA sequences can be modified by removing the  
5 non-coding nucleotides on the 5' and 3' ends of the  
coding region. The deleted non-coding nucleotides may or  
may not be replaced by other sequences known to be  
beneficial for expression. The transformation of these  
vectors into appropriate host cells as described above  
10 can produce desired heterodimers.

One skilled in the art could manipulate the  
sequences of Figures 1-6 by eliminating or replacing the  
mammalian regulatory sequences flanking the coding  
sequence with e.g., yeast or insect regulatory sequences,  
15 to create vectors for intracellular or extracellular  
expression by yeast or insect cells. [See, e.g.,  
procedures described in published European Patent  
Application 155,476] for expression in insect cells; and  
procedures described in published PCT application  
20 WO86/00639 and European Patent Application EPA 123,289  
for expression in yeast cells].

Similarly, bacterial sequences and preference  
codons may replace sequences in the described and  
exemplified mammalian vectors to create suitable  
25 expression systems for use in the production of BMP  
monomers in the method described above. For example, the  
coding sequences could be further manipulated (e.g.,

ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified BMP coding sequences could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al, Proc. Natl. Acad. Sci. USA, 77:5230-5233 (1980). The exemplary bacterial vector could then be transformed into bacterial host cells and BMP heterodimers expressed thereby. An exemplary vector for microbial, e.g., bacterial, expression is described below in Example 7.

Other vectors useful in the methods of this invention may contain multiple genes in a single transcription unit. For example, a proposed plasmid p7E2D contains the BMP-7 gene followed by the EMC leader sequence, followed by the BMP-2 gene, followed by the DHFR marker gene. Another example is plasmid p7E2ED which contains the BMP-7 gene, the EMC leader, the BMP-2 gene, another EMC leader sequence and the DHFR marker gene. Alternatively, the vector may contain more than one transcription unit. As one example, the plasmid p2ED7ED contains a transcription unit for BMP-2 and a separate transcription unit for BMP-7, i.e., BMP-2-EMC-DHFR and BMP-7-EMC-DHFR. Alternatively, each transcription unit on the plasmid may contain a different marker gene. For example, plasmid p2EN7ED contains BMP-2-EMC-Neo and BMP-7-EMC-DHFR.

Additionally the vectors also contain appropriate expression control sequences which are capable of directing the replication and expression of the BMP in the selected host cells. Useful regulatory sequences for such vectors are known to one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention. Similarly, the vectors may contain one or more selection markers, such as the antibiotic resistance gene, Neo or selectable markers such as DHFR and ADA. The presently preferred marker gene is DHFR. These marker genes may also be selected by one of skill in the art.

Once they are expressed by one of the methods described above, the heterodimers of this invention may be identified and characterized by application of a variety of assays and procedures. A co-precipitation (immunoprecipitation) assay may be performed with antibodies to each of the BMPs forming the heterodimer. Generally antibodies for this use may be developed by conventional means, e.g., using the selected BMP, fragments thereof, or synthetic BMP peptides as antigen. Antibodies employed in assays are generally polyclonal antibodies made from individual BMP peptides or proteins injected into rabbits according to classical techniques. This assay is performed conventionally, and permits the identification of the heterodimer, which is precipitated

by antibodies to both BMP components of the heterodimer.

In contrast, only one of the two antibodies causes precipitation of any homodimeric form which may be produced in the process of producing the heterodimer.

5 Another characterizing assay is a Western assay, employing a precipitating antibody, a probing antibody and a detecting antibody. This assay may also be performed conventionally, by using an antibody to one of the BMPs to precipitate the dimers, which are run on  
10 reducing SDS-PAGE for Western analysis. An antibody to the second BMP is used to probe the precipitates on the Western gel for the heterodimer. A detecting antibody, such as a goat-antirabbit antibody labelled with  
15 horseradish peroxidase (HRP), is then applied, which will reveal the presence of one of the component subunits of the heterodimer.

Finally, the specific activity of the heterodimer may be quantitated as described in detail in Example 6. Briefly, the amount of each BMP is  
20 quantitated using Western blot analysis or pulse labelling and SDS-PAGE analysis in samples of each BMP homodimer and the heterodimer. The W20 activity is also determined as described specifically in Example 8. The relative specific activities may be calculated by the  
25 formula: W20 alkaline phosphatase activity/amount of BMP on Western blot or by fluorography. As one example, this formula has been determined for the BMP-2/7 heterodimer,

demonstrating that the heterodimer has an estimated 5 to 50 fold higher specific activity than the BMP-2 homodimer.

The heterodimers of the present invention may have a variety of therapeutic and pharmaceutical uses, e.g., in compositions for wound healing, tissue repair, and in similar compositions which have been indicated for use of the individual BMPs. Increased potency of the heterodimers over the individual BMPs may permit lower dosages of the compositions in which they are contained to be administered to a patient in comparison to dosages of compositions containing only a single BMP. A heterodimeric protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a heterodimeric protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A heterodimeric protein of this invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an

environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Heterodimeric polypeptides of the invention may also be useful in the treatment of osteoporosis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g., European Patent Applications 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g., PCT Publication WO84/01106 incorporated by reference herein for discussion of wound healing and related tissue repair).

Additionally, the proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease in neuronal survival.

In view of the usefulness of the heterodimers, therefore, a further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. In addition, the invention comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of a heterodimeric protein of the invention in admixture

with a pharmaceutically acceptable vehicle, carrier or matrix. The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is  
5 within the skill of the art.

It is expected that the proteins of the invention may act in concert with other related proteins and growth factors. Therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of a heterodimeric protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned and concurrently filed U.  
10 S. applications described above. Such combinations may comprise separate molecules of the BMP proteins or other heteromolecules of the present invention.  
15

In further compositions, heterodimeric proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth  
20 factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses, in addition to  
25

humans, are desired patients for such treatment with heterodimeric proteins of the present invention.

The therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair.

Therapeutically useful agents other than the heterodimeric proteins of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the heterodimeric BMP composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the heterodimeric protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical

properties, cosmetic appearance and interface properties. The particular application of the heterodimeric BMP compositions will define the appropriate formulation. Potential matrices for the compositions may be

5 biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further

10 matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics.

15 Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

20

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applicatons, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the BMP compositions from dissassociating from the matrix.

25

The dosage regimen of a heterodimeric protein-containing pharmaceutical composition will be determined by the attending physician considering various factors which modify the action of the heterodimeric proteins, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the BMP proteins in the heterodimer and any additional BMP or other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

The following examples are illustrative of the present invention and do not limit its scope.

EXAMPLE 1 - BMP Vector Constructs and Cell Lines

A. BMP-2 Vectors

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) [Wong et al, Science, 228:810-815 (1985)] differing from the latter in that it

contains the ampicillin resistance gene (Amp) in place of the tetracycline resistance gene (Tet) and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described [R. J. Kaufman, Proc. Natl. Acad. Sci. USA, 82:689-693 (1985)] and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122, excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form. Plasmid pMT2 can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

Plasmid pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga et al, Biotechnology, 84:636 (1984)]. This removes bases 1075 to 1145 relative to the HindIII site near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

5' PO<sub>4</sub>-CATGGGCAGCTCGAG-3' (SEQ ID NO: 15)

at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease XhoI.

A derivative of pMT2 CXM, termed plasmid pMT23, 5 contains recognition sites for the restriction endonucleases PstI, EcoRI, SalI and XhoI.

Full length BMP-2 cDNA (Fig. 1) (SEQ ID NO: 1) is released from the λGT10 vector by digestion with EcoRI and subcloned into pSP65 [Promega Biotec, Madison, 10 Wisconsin; see, e.g., Melton et al, Nucl. Acids Res., 12:7035-7056 (1984)] in both orientations yielding pBMP-2 #39-3 or pBMP-2 #39-4.

The majority of the untranslated regions of the BMP-2 cDNA are removed in the following manner. The 5' 15 sequences are removed between the SalI site in the adapter (present from the original cDNA cloning) and the SalI site 7 base pairs upstream of the initiator ATG by digestion of the pSP65 plasmid containing the BMP-2 cDNA with SalI and religation. The 3' untranslated region is 20 removed using heteroduplex mutagenesis using the oligonucleotide

5' GAGGGTTGTGGGTGTCGCTAGTGAGTCGACTACAGCAAAATT 3'.

End SalI

(SEQ ID NO: 16)

25 The sequence contains the terminal 3' coding region of the BMP-2 cDNA, followed immediately by a recognition site for SalI. The sequence introduces a SalI site following the termination (TAG) codon.

The SalI fragment of this clone was subcloned into the expression vector pMT23, yielding the vector pMT23-BMP2 $\Delta$ UT. Restriction enzyme sites flank the BMP-2 coding region in the sequence PstI-EcoRI-SalI-BMP-2 cDNA-SalI-EcoRI-XhoI.

5 The expression plasmid pED4 [Kaufman et al, Nucl. Acids Res., 19:4485-4490 (1991)] was linearized by digestion with EcoRI and treated with calf intestinal phosphatase. The BMP-2 cDNA gene was excised from pMT23-BMP2 $\Delta$ UT by digestion with EcoRI and recovery of the 1.2 kb fragment by electrophoresis through a 1.0% low melt agarose gel. The linearized pED4 vector and the EcoRI BMP-2 fragment were ligated together, yielding the BMP-2 expression plasmid pBMP2 $\Delta$ -EMC.

10 15 Another vector pBMP-2 $\Delta$ -EN contains the same sequences contained within the vector pBMP2 $\Delta$ -EMC, except the DHFR gene has been replaced by conventional means with the neomycin resistance gene from the Tn5 transposable element.

20 B. BMP4 Vectors

A BMP-4 cDNA sequence set forth in Figure 2 (SEQ ID NO: 3), in which the 3' untranslated region is removed, is made via heteroduplex mutagenesis with the mutagenic oligonucleotide:

5' GGATGTGGGTGCCGCTGACTCTAGAGTCGACGGAATTC 3'  
End EcoRI  
(SEQ ID NO: 17)

This deletes all of the sequences 3' to the translation  
5 terminator codon of the BMP-4 cDNA, juxtaposing this  
terminator codon and the vector polylinker sequences.  
This step is performed in an SP65 vector [Promega  
Biotech] and may also be conveniently performed in pMT2-  
derivatives containing the BMP-4 cDNA similar to the BMP2-  
10 vectors described above. The 5' untranslated region is  
removed using the restriction endonuclease BsmI, which  
cleaves within the eighth codon of BMP-4 cDNA.

Reconstruction of the first eight codons  
is accomplished by ligation to oligonucleotides:

15 EcoRI Initiator BsmI  
5' AATTCACCATGATTCCTGGTAACCGAATGCT 3' (SEQ ID NO: 18)  
and

3' GTGGTACTAAGGACCATTGGCTTAC 5' (SEQ ID NO: 19)

These oligonucleotides form a duplex which has a BsmI  
20 complementary cohesive end capable of ligation to the  
BsmI restricted BMP-4 cDNA, and it has an EcoRI  
complementary cohesive end capable of ligation to the  
EcoRI restricted vector pMT2. Thus the cDNA for BMP-4  
with the 5' and 3' untranslated regions deleted, and  
25 retaining the entire encoding sequence is contained  
within an EcoRI restriction fragment of approximately 1.2  
kb.

The pMT2 CXM plasmid containing this BMP-4

sequence is designated pXMBMP-4 $\Delta$ UT. It is digested with EcoRI in order to release the BMP-4 cDNA containing insert from the vector. This insert is subcloned into the EcoRI site of the mammalian expression vector pED4, resulting pBMP4 $\Delta$ -EMC.

5

C. BMP-5 Vectors

A BMP-5 cDNA sequence comprising the nucleotide sequence from nucleotide #699 to #2070 of Fig. 5 (SEQ ID NO: 9) is specifically amplified as follows.

10 The oligonucleotides CGACCTGCAGCCACCATGCATCTGACTGTA (SEQ ID NO: 20) and TGCCTGCAGTTAACATTAGTGGCAGC (SEQ ID NO: 21) are utilized as primers to allow the amplification of nucleotide sequence #699 to #2070 of Fig. 5 from the BMP-5 insert of  $\lambda$ -ZAP clone U2-16 [ATCC #68109]. This

15 procedure introduces the nucleotide sequence CGACCTGCAGCCACC (SEQ ID NO: 22) immediately preceding nucleotide #699 and the nucleotide sequence CTGCAGGCA immediately following nucleotide #2070. The addition of these sequences results in the creation of PstI

20 restriction endonuclease recognition sites at both ends of the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease PstI and subcloned into the PstI site of the pMT2 derivative pMT21 [Kaufman, Nucl. Acids Res., 19:4485-4490 (1991)]. The resulting clone is

25 designated H5/5/pMT.

The insert of H5/5/pMT is excised by PstI

digestion and subcloned into the plasmid vector pSP65 [Promega Biotech] at the PstI site, resulting in plasmid BMP5/SP6. BMP5/SP6 and U2-16 are digested with the restriction endonucleases NsiI and NdeI to excise the portion of their inserts corresponding to nucleotides #704 to #1876 of Fig. 5. The resulting 1173 nucleotide NsiI-NdeI fragment of clone U2-16 is ligated into the NsiI-NdeI site of BMP5/SP6 from which the corresponding 1173 nucleotide NsiI-NdeI fragment had been removed. The resulting clone is designated BMP5mix/SP65.

Direct DNA sequence analysis of BMP5mix/SP65 is performed to confirm identity of the nucleotide sequences produced by the amplification to those set forth in Fig. 5. The clone BMP5mix/SP65 is digested with the restriction endonuclease PstI resulting in the excision of an insert comprising the nucleotides #699 to #2070 of Fig. 5 and the additional sequences containing the PstI recognition sites as described above. The resulting 1382 nucleotide PstI fragment is subcloned into the PstI site of the pMT2 derivative pMT21. This clone is designated BMP5mix/pMT21#2.

The same fragment is also subcloned into the PstI site of pED4 to yield the vector designated BMP5mix-EMC-11.

25           D.     BMP-6 Vectors

A BMP-6 cDNA sequence comprising the nucleotide sequence from nucleotide #160 to #1706 of

Fig. 4 (SEQ ID NO: 7) is produced by a series of techniques known to those skilled in the art. The clone BMP6C35 [ATCC 68245] is digested with the restriction endonucleases ApaI and TaqI, resulting in the excision of a 1476 nucleotide portion of the insert comprising nucleotide #231 to #1703 of Fig. 4. Synthetic oligonucleotides with SalI restriction endonuclease site converters are designed to replace those nucleotides corresponding to #160 to #230 and #1704 to #1706 which are not contained in the 1476 ApaI-TaqI fragment of the BMP-6 cDNA sequence.

Oligonucleotide/SalI converters conceived to replace the missing 5'  
(TCGACCCACCATGCCGGGGCTGGGGCGGAGGGCGCAGTGGCTGT  
15 GCTGGTGGTGGGGCTGTGCTGCAGCTGCTGCCGGGCC (SEQ ID NO: 23) and  
CGCAGCAGCTGCACAGCAGCCCCCACCACCAAGCACAGCCACTGCGCCCTCCGCCCA  
GCCCGGGCATGGTGGG) (SEQ ID NO: 24) and 3' (TCGACTGGTTT  
(SEQ ID NO: 25) and CGAAACCAG (SEQ ID NO: 26) ) sequences  
are annealed to each other independently. The annealed  
20 5' and 3' converters are then ligated to the 1476  
nucleotide ApaI-TaqI described above, creating a 1563  
nucleotide fragment comprising the nucleotide sequence  
from #160 to #1706 of Fig. 4 and the additional sequences  
contrived to create SalI restriction endonuclease sites  
25 at both ends. The resulting 1563 nucleotide fragment is  
subcloned into the SalI site of pSP64 [Promega Biotech,  
Madison, WI]. This clone is designated BMP6/SP64#15.

DNA sequence analysis of BMP6/SP64#15 is performed to confirm identity of the 5' and 3' sequences replaced by the converters to the sequence set forth in Fig. 4. The insert of BMP6/SP64#15 is excised by digestion with the restriction endonuclease SalI. The resulting 1563 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 and designated herein as BMP6/pMT21.

The PstI site of pED4 is converted to a SalI site by digestion of the plasmid with PstI and ligation to the converter oligonucleotides:

5'-TCGACAGGCTCGCCTGCA-3' (SEQ ID NO: 27) and  
3'-GTCCGAGCGG-5' (SEQ ID NO: 28).

The above 1563 nucleotide SalI fragment is also subcloned into the SalI site of this pED4 vector, yielding the expression vector BMP6/EMC.

#### E. BMP-7 Vectors

A BMP-7 sequence comprising the nucleotide sequence from nucleotide #97 to #1402 of Fig. 3 (SEQ ID NO: 5) is specifically amplified as follows. The oligonucleotides CAGGTCGACCCACCATGCACGTGCGCTCA (SEQ ID NO: 29) and TCTGTCGACCTCGGAGGGCTAGTGGC (SEQ ID NO: 30) are utilized as primers to allow the amplification of nucleotide sequence #97 to #1402 of Fig. 3 from the insert of clone PEH7-9 [ATCC #68182]. This procedure generates the insertion of the nucleotide sequence CAGGTCGACCCACC immediately preceding nucleotide #97 and

the insertion of the nucleotide sequence GTCGACAGA immediately following nucleotide #1402. The addition of these sequences results in the creation of a SalI restriction endonuclease recognition site at each end of 5 the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease SalI and subcloned into the SalI site of the plasmid vector pSP64 [Promega Biotech, Madison, WI] resulting in BMP7/SP6#2.

10 The clones BMP7/SP6#2 and PEH7-9 are digested with the restriction endonucleases NcoI and StuI to excise the portion of their inserts corresponding to nucleotides #363 to #1081 of Fig. 3. The resulting 719 nucleotide NcoI-StuI fragment of clone PEH7-9 is ligated 15 into the NcoI-StuI site of BMP7/SP6#2 from which the corresponding 719 nucleotide fragment is removed. The resulting clone is designated BMP7mix/SP6.

20 Direct DNA sequence analysis of BMP7mix/SP6 confirmed identity of the 3' region to the nucleotide sequence from #1082 to #1402 of Fig. 3, however the 5' region contained one nucleotide misincorporation.

25 Amplification of the nucleotide sequence (#97 to #1402 of Fig. 3) utilizing PEH7-9 as a template is repeated as described above. The resulting amplified DNA product of this procedure is digested with the restriction endonucleases SalI and PstI. This digestion results in the excision of a 747 nucleotide fragment

comprising nucleotide #97 to #833 of Fig. 3 plus the additional sequences of the 5' priming oligonucleotide used to create the SalI restriction endonuclease recognition site described earlier. This 747 SalI-PstI fragment is subcloned into a SalI-PstI digested pSP65 [Promega Biotech, Madison, WI] vector resulting in 5'BMP7/SP65. DNA sequence analysis demonstrates that the insert of the 5'BMP7/SP65#1 comprises a sequence identical to nucleotide #97 to #362 of Fig. 3.

The clones BMP7mix/SP6 and 5'BMP7/SP65 are digested with the restriction endonucleases SalI and NcoI. The resulting 3' NcoI-SalI fragment of BMP7mix/SP6 comprising nucleotides #363 to #1402 of Fig. 3 and 5' SalI-NcoI fragment of 5'BMP7/SP65 comprising nucleotides #97 to #362 of Fig. 3 are ligated together at the NcoI restriction sites to produce a 1317 nucleotide fragment comprising nucleotides #97 to #1402 of Fig. 3 plus the additional sequences derived from the 5' and 3' oligonucleotide primers which allows the creation of SalI restriction sites at both ends of this fragment.

This 1317 nucleotide SalI fragment is ligated into the SalI site of the pMT2 derivative pMT2Cla-2. pMT2Cla-2 is constructed by digesting pMT21 with EcoRV and XhoI, treating the digested DNA with Klenow fragment of DNA polymerase I and ligating ClaI linkers (NEBio Labs, CATCGATG). This removes bases 2171 to 2420 starting from the HindIII site near the SV40 origin of

replication and enhancer sequences of pMT2 and introduces a unique ClaI site, but leaves the adenovirus VAI gene intact, resulting in pMT2Cla-2. This clone is designated BMP-7-pMT2.

5           The insert of BMP-7-pMT2 is excised by digestion with the restriction endonuclease SalI. The resulting 1317 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 to yield the clone BMP-7/pMT21. This SalI fragment is also 10 subcloned into the SalI site of the pED4 vector in which the PstI site was converted into a SalI site as described above, resulting in the vector pBMP7/EMC#4.

F. BMP-8 Vectors

At present no mammalian BMP-8 vectors have 15 been constructed. However, using the sequence of Figure 6 (SEQ ID NO: 11), it is contemplated that vectors similar to those described above for the other BMPs may be readily constructed. A bacterial expression vector similar to the BMP-2 vector described in detail in Example 7 may also be constructed for BMP-8, by 20 introducing a Met before the amino acid #284 Ala of Fig. 6. This sequence of BMP-8 is inserted into the vector pALBP2-781 in place of the BMP-2 sequence. See Example 7.

25           G. BMP Vectors Containing the Adenosine Deaminase (Ada) Marker

BMP genes were inserted into the vector

pMT3SV2Ada [R. J. Kaufman, Meth. Enz., 185:537-566 (1990)] to yield expression plasmids containing separate transcription units for the BMP cDNA gene and the selectable marker Ada. pMT3SV2Ada contains a polylinker with recognition sites for the enzymes PstI, EcoRI, SalI and XbaI that can be used for insertion of and expression of genes (i.e. BMP) in mammalian cells. In addition, the vector contains a second transcription unit encoding Ada which serves as a dominant and amplifiable marker in mammalian cells.

To construct expression vectors for BMP-5, BMP-6 and BMP-7, individually, the same general method was employed. The gene for BMP 5 (Fig. 5), 6 (Fig. 4) or 7 (Fig. 3) was inserted into the polylinker essentially as described above for the pED4 vector. These vectors can be used for transfection into CHO DUKX cells and subsequent selection and amplification using the Ada marker as previously described [Kaufman et al, Proc. Natl. Acad. Sci. USA, 83:3136-3140 (1986)]. Since each such vector does not contain a DHFR gene, the resultant transformed cells remain DHFR negative and can be subsequently transfected with a second vector containing a different BMP in conjunction with DHFR and amplified with methotrexate.

Alternatively, the pMT3SV2Ada/BMP vectors can be used to transfect stable CHO cell lines previously transfected with a different BMP gene and amplified using

the DHFR/methotrexate system. The resultant transfectants can be subsequently amplified using the Ada system, yielding cell lines that coexpress two different BMP genes, and are amplified using both the DHFR and Ada markers.

5

#### H. BMP-Expressing Mammalian Cell Lines

At present, the most desirable mammalian cell lines for use in producing the recombinant homodimers and heterodimers of this invention are the following. These cell lines were prepared by conventional transformation of CHO cells using vectors described above.

The BMP-2 expressing cell line 2EG5 is a CHO cell stably transformed with the vector pBMP2delta-EMC.

15

The BMP-4 expressing cell line 4E9 is a CHO cell stably transformed with the vector pBMP4delta-EMC.

20

The BMP-5 expressing cell line 5E10 is a CHO cell stably transformed with the vector BMP5mix-EMC-11 (at a amplification level of 2 micromolar MTX).

The BMP-6 expressing cell line 6HG8 is a CHO cell stably transformed with the vector BMP6/EMC.

25

The BMP-7 expressing cell line 7MB9 is a CHO cell stably transformed with the vector BMP7/pMT21.

EXAMPLE 2 - TRANSIENT EXPRESSION OF BMP HETERODIMERS

The heterodimers of the present invention may be prepared by co-expression in a transient expression system for screening in the assays of Example 8 by two different techniques as follows.

5 In the first procedure, the pMT2-derived and EMC-derived expression plasmids described in Example 1 and other similarly derived vectors were constructed which encoded, individually, BMP-2 through BMP-7, and transforming growth factor-beta (TGF $\beta$ 1). All  
10 combinations of pairs of plasmids were mixed in equal proportion and used to co-transfect CHO cells using the DEAE-dextran procedure [Sompayrac and Danna, Proc. Natl. Acad. Sci. USA, 78:7575-7578 (1981); Luthman and Magnusson, Nucl. Acids Res., 11:1295-1308 (1983)]. The  
15 cells are grown in alpha Minimal Essential Medium ( $\alpha$ -MEM) supplemented with 10% fetal bovine serum, adenosine, deoxyadenosine, thymidine (100  $\mu$ g/ml each), pen/strep, and glutamine (1 mM).

20 The addition of compounds such as heparin, suramin and dextran sulfate are desirable in growth medium to increase the amounts of BMP-2 present in the conditioned medium of CHO cells. Similarly responsive to such compounds is BMP-5. Therefore, it is expected that these compounds will be added to growth medium for any  
25 heterodimer containing these BMP components. Other BMPs may also be responsive to the effects of these compounds, which are believed to inhibit the interaction of the

mature BMP molecules with the cell surface.

The following day, fresh growth medium, with or without 100 µg/ml heparin, was added. Twenty-four hours later, conditioned medium was harvested.

5           In some experiments, the conditioned medium was collected minus heparin for the 24-48 hour period post-transfection, and the same plates were then used to generate conditioned medium in the presence of heparin 48-72 hour post-transfection. Controls included  
10          transfected cells with expression plasmids lacking any BMP sequences, transfected cells with plasmids containing sequences for only a single BMP, or mixing conditioned medium from cells transfected with a single BMP with conditioned medium from cells transfected with a  
15          different BMP.

Characterizations of the coexpressed heterodimer BMPs in crude conditioned media, which is otherwise not purified, provided the following results. Transiently coexpressed BMP was assayed for induction of alkaline phosphatase activity on W20 stromal cells, as described in Example 8.  
20

25          Co-expression of BMP-2 with BMP-5, BMP-6 and BMP-7, and BMP-4 with BMP-5, BMP-6 and BMP-7 yielded more alkaline phosphatase inducing activity in the W20 assay than either of the individual BMP homodimers alone or mixtures of homodimers, as shown below. Maximal activity (in vitro), was obtained when BMP-2 was coexpressed with

BMP-7. Increased activity was also found the heterodimers BMP-2/5; BMP-2/6; BMP-4/5; BMP-4/6; and BMP-4/7.

	Condition Medium						
	TGF- $\beta$	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	33	240	99	89	53	9	29
BMP-3	-	-	-	--	14	-	
BMP-4	12	115	25	22	24		
BMP-5	-	-	--	--			
BMP-6	-	-	-				
BMP-7	-	-					
TGF- $\beta$	-						

	Condition Medium + heparin						
	TGF- $\beta$	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	88	454	132	127	70	77	169
BMP-3	-	-	-	-	7	-	
BMP-4	7	119	30	41	37		
BMP-5	-	-	--	--			
BMP-6	-	-	--				
BMP-7	-	-					
TGF- $\beta$	-						

Units: 1 unit of activity is equivalent to that of 1 ng/ml of rhBMP-2.

--: indicates activity below the detection limit of the assay.

These BMP combinations were subsequently expressed  
5 using various ratios of expression plasmids (9:1, 3:1,  
1:1, 1:3, 1:9) during the CHO cell transient  
transfection. The performance of this method using  
plasmids containing BMP-2 and plasmids containing BMP-7  
at plasmid number ratios ranging from 9:1 to 1:9,  
10 respectively, demonstrated that the highest activity in

the W20 assay was obtained when approximately the same number of plasmids of each BMP were transfected into the host cell. Ratios of BMP-2 to BMP-7 plasmids of 3:1 to 1:3, respectively, also resulted in increased activity in 5 W20 assay in comparison to host cells transfected with plasmids containing only a single BMP. However, these latter ratios produced less activity than the 1:1 ratio.

Similar ratios may be determined by one of skill in the art for heterodimers consisting of other 10 than BMP-2 and BMP-7. For example, preliminary work on the heterodimer formed between BMP-2 and BMP-6 has indicated that a preferred ratio of plasmids for co-transfection is 3:1, respectively. The determination of preferred ratios for this method is within the skill of 15 the art.

As an alternative means to transiently generate coexpressed BMPs, the stable CHO cell lines identified in Example 1 expressing each BMP-2, BMP-4, BMP-5, BMP-6 and BMP-7, are cocultured for one day, and are then fused 20 with 46.7% polyethylene glycol (PEG). One day post-fusion, fresh medium is added and the heterodimers are harvested 24 hours later for the W20 assay, described in Example 8. The assay results were substantially similar to those described immediately above.

Therefore, all combinations of BMP-2 or 4 25 coexpressed with either BMP-5, 6 or 7 yielded greater activity than any of the BMP homodimers alone. In

control experiments where each BMP homodimer was expressed alone and conditioned media mixed post harvest, the activity was always intermediate between the individual BMPs, demonstrating that the BMP co-expressed heterodimers yield higher activity than combinations of the individually expressed BMP homodimers.

EXAMPLE 3 - STABLE EXPRESSION OF BMP HETERODIMERS

A. BMP-2/7

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-7.

A preferred stable cell line, 2E7E-10, was obtained as follows: Plasmid DNA (a 1:1 mixture of pBMP-7-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 2E7E, is carried out up to a concentration of 0.5  $\mu$ M MTX.

The cell line is then subcloned and assayed for heterodimer 2/7 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 2E7E-10. This cell line secretes BMP-2/7 heterodimer proteins into the media containing 0.5 µM MTX.

The CHO cell line 2E7E-10 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 2E7E-10 preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at least 5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell

line, the stable CHO cell line 7MB9, previously transfected with pBMP-7-pMT2, and which expresses BMP-7, is employed. 7MB9 may be amplified and selected to 2  $\mu$ M methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 7MB9 is transfected with the expression vector pBMP-2 $\Delta$ -EN (EMC-Neo) containing BMP-2 and the neomycin resistance gene from the Tn5 transposable element. The resulting transfected stable cell line was selected for both G-418 and MTX resistance. Individual clones were picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced activity by transient coexpression will likewise yield greater activity upon stable expression.

15           B. BMP-2/6

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-6.

20           A preferred stable cell line, 12C07, was obtained as follows: Plasmid DNA (a 1:3 mixture of pBMP-6-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

25           Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are

counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 12-C, is carried out up to a concentration of 2.0  $\mu$ M MTX. The cell line is then subcloned and assayed for heterodimer 2/6 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 12C07. This cell line secretes BMP-2/6 heterodimer proteins into the media containing 2.0  $\mu$ M MTX.

The CHO cell line 12C07 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 12C07

preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at 5 least 3-5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell line, the stable CHO cell line 2EG5, previously transfected with pBMP-2-EMC, and which expresses BMP-2, 10 is employed. 2EG5 may be amplified and selected to 2  $\mu$ M methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 2EG5 is transfected with the expression vector pBMP-6-ada (ada deaminase) containing BMP-6 and the ADA resistance gene. 15 The resulting transfected stable cell line was selected for both DCF and MTX resistance. Individual clones are picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced 20 activity by transient coexpression will likewise yield greater activity upon stable expression.

EXAMPLE 4-PURIFICATION OF BMP2/7 AND BMP-2/6 HETERODIMER

The same purification procedure is used for BMP-2/6 25 heterodimer and BMP-2/7 heterodimer. Conditioned media from cultures of cell line 2E7E-10 or 12C07 containing

recombinantly produced BMP heterodimer 2/7V or 2/6,  
respectively, can be generated from either adherent or  
suspension cultures. For small to medium scale  
generation of coexpressed BMP, adherent cultures are  
seeded into roller bottles and allowed to grow to  
confluence in alpha-Minimal Eagles Medium [ $\alpha$ -MEM, Gibco,  
Grand Island, NY] containing 10% dialyzed heat-  
inactivated fetal calf serum [Hazleton, Denver, PA]. The  
media is then switched to a serum-free, albumin free, low  
protein medium based on a 50:50 mixture of Delbecco's  
Modified Eagle's medium and Hams F-12 medium, optionally  
supplemented with 100 micrograms/ml dextran sulfate.  
Four or five daily harvests are pooled, and used to  
purify the recombinant protein.

Conditioned medium from roller bottle cultures  
obtained as described above was thawed slowly at room  
temperature and pooled. The pH of the pooled medium was  
adjusted to pH 8.0 using 1 M Tris, pH 8.0. A column was  
poured containing Matrex Cellufine Sulfate [Amicon] and  
equilibrated in 50 mM Tris, pH 8.0.

Upon completion of loading of the medium, the  
column was washed with buffer containing 50 mM Tris, 0.4  
M NaCl, pH 8.0 until the absorbance at 280 nm reached  
baseline. The column was then washed with 50 mM Tris, pH  
8.0 to remove NaCl from the buffer. The resin was then  
washed with 50 mM Tris, 0.2 M NaCl, 4 M Urea, pH 8.0  
until a peak had eluted. The column was then washed into

50 mM Tris, pH 8.0 to remove the urea.

The bound BMP-2/7 or BMP-2/6 was then eluted using 50 mM Tris, 0.5 M NaCl, 0.5 M Arginine, pH 8.0. The eluate was collected as a single pool and may be 5 optionally stored frozen prior to further purification. This Cellufine Sulfate eluate was diluted with 14 volumes of 6M urea and the pH of the sample was then adjusted to 6.0. A hydroxyapatite-Ultrogel [IBF] column was poured and equilibrated with 80 mM potassium phosphate, 6M urea, 10 pH 6.0.

After the completion of sample loading, the column was washed with 10 bed volumes of the equilibration buffer. Bound BMP-2/7 or BMP-2/6 heterodimers were eluted with 5 bed volumes of 100 mM potassium phosphate, 6M urea, pH 7.4. This eluate was 15 loaded directly onto a Vydac C<sub>4</sub> reverse-phase HPLC column equilibrated in water - 0.1% TFA. BMP-2/7 or BMP-2/6 heterodimers were eluted with a gradient of 30-50% acetonitrile in water - 0.1% trifluoroacetic acid.

20 Fractions containing BMPs are identified by SDS-PAGE in the presence or absence of reductant. The identity of the BMPs with respect to the heterodimers vs. homodimers is determined by 2D-PAGE (+/- reductant). Fractions with heterodimers gave bands which reduce to two spots. Bands 25 from homodimer fractions reduce to a single spot for each BMP species.

The BMP-2/6 heterodimer subunits are analyzed on a protein sequenator. BMP-2/6 heterodimers of the following species are present: BMP-6 subunit beginning with amino acid #375 Ser-Ala-Ser-Ser in association with BMP-2 subunit beginning with amino acid #283 Gin-Ala-Lys or #249 Ser-Lev-His, though other less abundant species may be present.

It is contemplated that the same or substantially similar purification techniques may be employed for any recombinant BMP heterodimer of this invention. The hydroxyapatite-Ultrogel column may be unnecessary and that the purification scheme may be modified by loading the Cellufine Sulfate eluate directly onto the C<sub>4</sub> reverse-phase HPLC column without use of the former column for BMP2/7 or BMP-2/6 or the other heterodimers of this invention.

EXAMPLE 5 - PROTEIN CHARACTERIZATION

Total protein secreted from the co-expressing cell lines is analyzed after labelling with <sup>35</sup>S-methionine or by Western blot analysis using antibodies raised against both BMPs of the heterodimer, e.g., BMP-2 and BMP-7. Together with the alkaline phosphatase assays, the data indicates the presence of the heterodimer and the specific activity. The following specific details are directed towards data collected for the BMP-2/7 and BMP-2/6 heterodimers; however, by application of similar

methods to the other heterodimers described herein,  
similar results are expected.

A. <sup>35</sup>S-Met labelling

Cell lines derived by cotransfection of  
5 BMP2<sub>A</sub>-EMC and BMP7<sub>A</sub>-EMC expression vectors were pulsed  
with <sup>35</sup>S-methionine for 15 minutes, and chased for 6 hours  
in serum free media in the presence or absence of  
heparin. Total secreted protein was analyzed under  
reducing conditions by PAGE and fluorography. The  
10 results demonstrate that several cell lines secrete both  
BMP-2 and BMP-7 protein. There is a good correlation  
between the amount of alkaline phosphatase activity and  
the amount of coexpressed protein.

Several cell lines secrete less total BMP-  
15 2 and 7 than the BMP-2-only expressing cell line 2EG5,  
which produces 10 µg/ml BMP-2. Cell line 2E7E-10  
(amplified at a level of 0.5mM MTX) secretes equal  
proportions of BMP-2 and BMP-7 at about the same overall  
level of expression as the cell line 2EG5. Cell line  
20 2E7E-10 produces the equivalent of 600 micrograms/ml of  
BMP-2 homodimer activity in one assay.

Total labelled protein was also analyzed on a  
two-dimensional non-reducing/reducing gel system to  
ascertain whether a heterodimer is made. Preliminary  
25 results demonstrate the presence of a unique spot in this  
gel system that is not found in either the BMP-2-only or  
BMP-7-only cell lines, suggesting the presence of 2/7

heterodimer. The same gel with purified material produced the same results (e.g., two unique spots on the gel) indicative of the presence of the 2/7 heterodimer. The homodimer of BMP2 produced distinct species on this 5 gel system.

In contrast to the recombinant BMP-2/7 purification, BMP-2 homodimers are not detected during the BMP-2/6 preparation; however, significant amounts of BMP-6 homodimers are found. In addition, a significant amount 10 of a -20 amino acid N-terminal truncated form of BMP-6 is found; this could be eliminated by the inclusion of protease inhibitors during cell culture. BMP-2/6 was found to elute two to three fractions later from C4 RP-HPLC than did BMP-2/7.

15 Amino acid sequencing indicates that the predominant BMP-2/7 heterodimer species comprises a mature BMP-2 subunit [amino acid #283(Gln)-#396(Arg)] and a mature subunit of BMP-7 [#293(Ser)-#431(His)]. BMP-2/6 heterodimer comprises the mature BMP-2 subunit (#283-396) 20 and the mature BMP-6 subunit [#375(Ser)-#513(His)].

B. Immunoprecipitation coupled to Western blot analysis

Conditioned media from a BMP-2-only (2EG5), a BMP-7-only (7MB9), or the 2E7E-10 co-expressing cell line were subjected to immunoprecipitation with either a BMP-2 or BMP-7 antibody (both conventional 25

polyclonal antibodies raised in rabbits), then analyzed on Western blots probed with either an anti-BMP-2 or anti-BMP-7 antibody. The 2/7 heterodimer precipitates and is reactive on Western blots with both the BMP-2 and 5 BMP-7 antibodies, while either BMP by itself reacts with its specific antibody, but not with the reciprocal antibody.

It has been demonstrated using this strategy that a protein in the co-expressing cell line 10 that is precipitated by the anti-BMP-7 antibody W33 [Genetics Institute, Inc, Cambridge, Massachusetts] and reacts on a Western blot with the anti-BMP-2 antibody W12 or W10 [Genetics Institute, Inc.] is not present in the BMP-2 or 7-only expressing cell lines. This experiment 15 indicates that this protein species is the heterodimeric protein. Conversely, precipitation with W12 and probing with W33 yielded similar results.

#### EXAMPLE 6 - SPECIFIC ACTIVITY OF HETERODIMERS

##### A. In vitro Assays

20 The specific activity of the BMP-2/7 or BMP-2/6 heterodimer and the BMP-2 homodimer secreted into growth medium of the stable cell lines 2E7E-10 and 2EG55, and 12C07 and 2EG5, respectively, were estimated as follows.

25 The amount of BMP protein in conditioned medium was measured by either Western blot analysis or by

analyzing protein secreted from  $^{35}$ S-methionine labelled cells by PAGE and fluorography. The amount of activity produced by the same cell lines on W20 cells using either the alkaline phosphatase assay or osteocalcin-induction assay was then estimated. The specific activity of the BMP was calculated from the ratio of activity to protein secreted into the growth medium.

In one experiment 2E7E-10 and 2EG5 secreted similar amounts of total BMP proteins as determined by PAGE and fluorography. 2E7E-10 produced about 50-fold more alkaline phosphatase inducing activity than the 2EG5, suggesting that the specific activity of the heterodimer is about 50-fold higher than the homodimer.

In another experiment the amount of BMP-2 secreted by 2EG5 was about 50% higher than BMP-2/7 secreted by 2E7E-10, however, 2E7E-10 produced about 10-fold more osteocalcin-inducing activity than 2EG5. From several different experiments of this type the specific activity of the BMP-2/7 heterodimer is estimated to be between 5 to 50 fold higher than the BMP-2 homodimer.

Figures 8 and 9 compare the activity of BMP-2 and BMP-2/7 in the W20 alkaline phosphatase and BGP (Bone Gla Protein, osteocalcin) assays. BMP-2/7 has greatly increased specific activity relative to BMP-2 (Figure 8). From Figure 8, approximately 1.3 ng/ml of BMP-2/7 was sufficient to induce 50% of the maximal alkaline phosphatase response in W-20 cells. A comparable value

for BMP-2 is difficult to calculate, since the alkaline phosphatase response did not maximize, but greater than 30 ng/ml is needed for a half-maximal response. BMP-2/7 thus has a 20 to 30-fold higher specific activity than 5 BMP-2 in the W-20 assay.

As seen in Figure 9, BMP-2/7 was also a more effective stimulator of BGP (bone gla protein, osteocalcin) production than BMP-2 in this experiment. Treating W-20-17 cells with BMP-2/7 for four days 10 resulted in a maximal BGP response with 62 ng/ml, and 11 ng/ml elicits 50% of the maximal BGP response. In contrast, maximal stimulation of BGP synthesis by BMP-2 was not seen with doses up to 468 ng/ml of protein. The minimal dose of BMP-2/7 needed to elicit a BGP response 15 by W-20-17 cells was 3.9 ng/ml, about seven-fold less than the 29 ng/ml required of BMP-2. These results were consistent with the data obtained in the W-20-17 alkaline phosphatase assays for BMP-2 and BMP-2/7.

Preliminary analysis indicates that BMP-2/6 has 20 a specific activity in vitro similar to that of BMP-2/7. The potencies of BMP-2 and BMP-2/6 on induction of alkaline phosphatase production in W-20 is compared, as shown in Figure 12, BMP-2/6 has a higher specific 25 activity than BMP-2 in this assay system. This data is in good agreement with data obtained from the *in vivo* assay of BMP-2 and BMP-2/6).

B. In Vivo Assay

(i) BMP-2/7

The purified BMP-2/7 and BMP-2 were tested in  
the rat ectopic bone formation assay. A series of  
5 different amounts of BMP-2/7 or BMP-2 were implanted in  
triplicate in rats. After 5 and 10 days, the implants  
were removed and examined histologically for the presence  
of bone and cartilage. The histological scores for the  
amounts of new cartilage and bone formed are summarized  
10 in Table A.

Table A

		5 Day Implants		10 Day Implants	
		BMP-2/7	BMP-2	BMP-2/7	BMP-2
0.04	C	± - ±	---	± - ±	---
	B	---	---	± - ±	---
0.02	C	± 1 ±	---	2 1 2	- ± ±
	B	---	---	1 ± 1	- ± -
1.0	C	1 ± ±	± ± ±	2 2 2	1 1 ±
	B	---	---	2 3 3	1 1 ±
5.0	C	2 2 1	1 ± 1	1 1 2	1 2 1
	B	± - 1	---	4 4 3	2 3 2
25.0	C			± ± 2	2 2 2
	B			4 4 3	3 3 3

The amount of BMP-2/7 required to induce cartilage and bone in the rat ectopic assay is lower than that of BMP-2. Histologically, the appearance of cartilage and bone induced by BMP-2/7 and BMP-2 are identical.

5                   (ii) BMP-2/6

The *in vivo* activity of BMP-2/6 was compared with that of BMP-2 by implantation of various amounts of each BMP for ten days in the rat ectopic bone formation assay. The results of this study (Table B, Figure 13) indicate 10 that BMP-2/6, similar to BMP-2/7, has increased *in vivo* activity relative to BMP-2. The specific activities of BMP-2, BMP-6, and BMP-2/6 are compared in the ectopic bone formation assay ten days after the proteins are implanted. The results of these experiments are shown in 15 Table C and Figure 14. BMP-2/6 is a more potent inducer of bone formation than either BMP-2 or BMP-6. The amount

of bone formation observed with BMP-2/6 was comparable to that observed with equivalent doses of BMP-2/7. The appearance of BMP-2/6 implants is quite similar to implants containing BMP-2 or BMP-2/7.

5

**Table B**  
**Histological scores of Implants of BMP 2/6 and BMP-2 In rat ectopic assay (10 day implants).**

	BMP ( $\mu$ g)	C/B	BMP-2/6	BMP-2
10	0.04	C	- ± -	- - -
		B	- - -	- - -
	0.20	C	1 1 ±	- - -
		B	± ± ±	- - -
	1.0	C	1 3 3	1 1 ±
		B	1 2 2	1 1 ±
25.	5.0	C	2 2 2	1 2 2
		B	2 3 3	2 2 2
25.		C	1 1 1	2 2 1
		B	3 3 3	3 3 3

15

**Table C**  
**Histological scores of implants of BMP-2, BMP-6, and BMP-2/6 in rat ectopic assay (10 day implants).**

	BMP ( $\mu$ g)	C/B	BMP-2	BMP-6	BMP-2/6
20	0.04	C	- - -	- - -	- - ±
		B	- - -	- - -	- - ±
	0.20	C	- - 2	- - -	1 2 2
		B	- - 1	- - -	2 2 2
	1.0	C	- ± ±	2 1 1	1 1 1
		B	- ± ±	1 ± ±	3 3 2
25.	5.0	C	2 2 1	3 1 3	± ± 1
		B	1 1 1	2 ± 1	4 5 4
25.		C	± ± ±	± ± ±	± ± ±
		B	5 4 5	4 4 5	4 5 3

25

EXAMPLE 7 - EXPRESSION OF BMP DIMER IN E. COLI

A biologically active, homodimeric BMP-2 was

expressed in E. coli using the techniques described in

European Patent Application 433,255 with minor modifications. Other methods disclosed in the above-referenced European patent application may also be employed to produce heterodimers of the present invention from E. coli. Application of these methods to the heterodimers of this invention is anticipated to produce active BMP heterodimeric proteins from E. coli.

5           A.    BMP-2 Expression Vector

An expression plasmid pALBP2-781 (Figure 10 7) (SEQ ID NO: 13) was constructed containing the mature portion of the BMP-2 (SEQ ID NO: 14) gene and other sequences which are described in detail below. This plasmid directed the accumulation of 5-10% of the total cell protein as BMP-2 in an E. coli host strain, GI724, 15 described below.

Plasmid pALBP2-781 contains the following 20 principal features. Nucleotides 1-2060 contain DNA sequences originating from the plasmid pUC-18 [Norrrander et al, Gene, 26:101-106 (1983)] including sequences containing the gene for  $\beta$ -lactamase which confers resistance to the antibiotic ampicillin in host E. coli strains, and a colE1-derived origin of replication.

Nucleotides 2061-2221 contain DNA sequences for the major leftward promoter (pL) of bacteriophage  $\lambda$  [Sanger et al, 25 J. Mol. Biol., 162:729-773 (1982)], including three operator sequences, O<sub>L</sub>1, O<sub>L</sub>2 and O<sub>L</sub>3. The operators are the binding sites for  $\lambda$ cI repressor protein,

intracellular levels of which control the amount of transcription initiation from pL. Nucleotides 2222-2723 contain a strong ribosome binding sequence included on a sequence derived from nucleotides 35566 to 35472 and 5 38137 to 38361 from bacteriophage lambda as described in Sanger et al, J. Mol. Biol., 162:729-773 (1982). Nucleotides 2724-3133 contain a DNA sequence encoding mature BMP-2 protein with an additional 62 nucleotides of 10 3'-untranslated sequence.

Nucleotides 3134-3149 provide a "Linker" DNA sequence containing restriction endonuclease sites. Nucleotides 3150-3218 provide a transcription termination sequence based on that of the E. coli aspA gene [Takagi et al, Nucl. Acids Res., 13:2063-2074 (1985)]. 15 Nucleotides 3219-3623 are DNA sequences derived from pUC-18.

As described below, when cultured under the appropriate conditions in a suitable E. coli host strain, pALBP2-781 can direct the production of high 20 levels (approximately 10% of the total cellular protein) of BMP-2 protein.

pALBP2-781 was transformed into the E. coli host strain GI724 (F, lacI<sup>q</sup>, lacP<sup>L8</sup>, ampC::λcI<sup>+</sup>) by the procedure of Dagert and Ehrlich, Gene, 6:23 (1979). [The 25 untransformed host strain E. coli GI724 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland on January 31, 1991 under ATCC

No. 55151 for patent purposes pursuant to applicable laws and regulations.] Transformants were selected on 1.5% w/v agar plates containing IMC medium, which is composed of M9 medium [Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory, New York (1972)] supplemented with 0.5% w/v glucose, 0.2% w/v casamino acids and 100 µg/ml ampicillin.

GI724 contains a copy of the wild-type  $\lambda$ cI repressor gene stably integrated into the chromosome at the ampC locus, where it has been placed under the transcriptional control of Salmonella typhimurium trp promoter/operator sequences. In GI724,  $\lambda$ cI protein is made only during growth in tryptophan-free media, such as minimal media or a minimal medium supplemented with casamino acids such as IMC, described above. Addition of tryptophan to a culture of GI724 will repress the trp promoter and turn off synthesis of  $\lambda$ cI, gradually causing the induction of transcription from pL promoters if they are present in the cell.

GI724 transformed with pALBP2-781 was grown at 37°C to an  $A_{550}$  of 0.5 (Absorbence at 550 nm) in IMC medium. Tryptophan was added to a final concentration of 100 µg/ml and the culture incubated for a further 4 hours. During this time BMP-2 protein accumulated to approximately 10% of the total cell protein, all in the "inclusion body" fraction.

BMP-2 is recovered in a non-soluble,

monomeric form as follows. Cell disruption and recovery is performed at 4°C. Approximately 9 g of the wet fermented E. coli GI724/pALBP2-781 cells are suspended in 30 mL of 0.1 M Tris/HCl, 10 mM EDTA, 1 mM phenyl methyl sulphonyl fluoride (PMSF), pH 8.3 (disruption buffer).  
5 The cells are passed four times through a cell disrupter and the volume is brought to 100 mL with the disruption buffer. The suspension is centrifuged for 20 min.  
(15,000 x g). The pellet obtained is suspended in 50 mL  
10 disruption buffer containing 1 M NaCl and centrifuged for 10 min. as above. The pellet is suspended in 50 mL disruption buffer containing 1% Triton X-100 (Pierce) and again centrifuged for 10 min. as above. The washed pellet is then suspended in 25 mL of 20 mM Tris/HCl, 1 mM  
15 EDTA, 1 mM PMSF, 1% DTT, pH 8.3 and homogenized in a glass homogenizer. The resulting suspension contains crude monomeric BMP-2 in a non-soluble form.

Ten mL of the BMP-2 suspension, obtained as described above, are acidified with 10% acetic acid to pH 2.5 and centrifuged in an Eppendorf centrifuge for 10 min. at room temperature. The supernatant is chromatographed. Chromatography was performed on a Sephadryl S-100 HR column (Pharmacia, 2.6 x 83 cm) in 1% acetic acid at a flow rate of 1.4 mL/minute. Fractions containing monomeric, BMP-2 are pooled. This material is  
25 used to generate biologically active, homodimer BMP-2.

Biologically active, homodimeric BMP-2 can

be generated from the monomeric BMP-2 obtained following solubilization and purification, described above, as follows.

5           0.1, 0.5 or 2.5 mg of the BMP-2 is dissolved at a concentration of 20, 100 or 500 µg/mL, respectively, in 50 mM Tris/HCl, pH 8.0, 1 M NaCl, 5 mM EDTA, 2 mM reduced glutathione, 1 mM oxidized glutathione and 33 mM CHAPS [Calbiochem]. After 4 days at 4°C or 23°C, the mixture is diluted 5 to 10 fold with 0.1% TFA.

10           Purification of biologically active BMP-2 is achieved by subjecting the diluted mixture to reverse phase HPLC on a Vydac C4 214TP54 column (25 x .46 cm) [The NEST Group, USA] at a flow rate of 1 ml/minute. Buffer A is 0.1% TFA. Buffer B is 90% acetonitrile, and 15       0.1% TFA. The linear gradient was 0 to 5 minutes at 20% Buffer B; 5 to 10 minutes at 20 to 30 % Buffer B; 10 to 40 minutes at 30 to 60% Buffer B; and 40 to 50 minutes at 60 to 100% Buffer B. Homodimeric BMP-2 is eluted and collected from the HPLC column.

20           The HPLC fractions are lyophilized to dryness, redissolved in sample buffer (1.5 M Tris-HCl, pH 8.45, 12% glycerol, 4% SDS, .0075% Serva Blue G, .0025% Phenol Red, with or without 100 mM dithiothreitol) and heated for five minutes at 95°C. The running buffer is 25       100 mM Tris, 100 mM tricine (16% tricine gel) [Novex], 0.1% SDS at pH 8.3. The SDS-PAGE gel is run at 125 volts for 2.5 hours.

The gel is stained for one hour with 200 ml of 0.5% Coomassie Brilliant Blue R-250, 25% isopropanol, 10% acetic acid, heated to 60°C. The gel is then destained with 10% acetic acid, 10% isopropanol until the background is clear.

5 The reduced material ran at approximately 13kD; the non-reduced material ran at approximately 30 kD, which is indicative of the BMP-2 dimer. This material was later active in the W20 assay of Example 8.

10 B. BMP-7 Expression Vector

For high level expression of BMP-7 a plasmid pALBMP7-981 was constructed. pALBMP7-981 is identical to plasmid pALBP2-781 with two exceptions: the BMP-2 gene (residues 2724-3133 of pALBP2-781) is replaced by the mature portion of the BMP-7 gene, deleted for sequenced encoding the first seven residues of the mature BMP-7 protein sequence:

	ATGTCTCATAATC	GTTCTAAAAC	TCCAAAAAAAT	CAAGAAGCTC	TGCGTATGGC
	CAACGTGGCA	GAGAACAGCA	GCAGCGACCA	GAGGCAGGCC	TGTAAGAACG
	ACGAGCTGTA	TGTCAGCTTC	CGAGACCTGG	GCTGGCAGGA	CTGGATCATC
	GCGCCTGAAG	GCTACGCCGC	CTACTACTGT	GAGGGGGAGT	GTGCCTTCCC
5	TCTGAACTCC	TACATGAACG	CCACCAACCA	CGCCATCGTG	CAGACGCTGG
	TCCACTTCAT	CAACCCGGAA	ACGGTGCCCA	AGCCCTGCTG	TGCGCCCACG
	CAGCTCAATG	CCATCTCCGT	CCTCTACTTC	GATGACAGCT	CCAACGTCAT
	CCTGAAGAAA	TACAGAAACA	TGGTGGTCCG	GGCCTGTGGC	TGCCACTAGC
	TCCTCCGAGA	ATTCA GACCC	TTTGGGGCCA	AGTTTTCTG	GATCCT

10 and the ribosome binding site found between residues  
 2707 and 2723 in pALBP2-781 is replaced by a different  
 ribosome binding site, based on that found preceding the  
 T7 phage gene 10, of sequence 5'-CAAGAAGGAGATATACAT-3'.  
 The host strain and growth conditions used for the  
 15 production of BMP-7 were as described for BMP-2.

C. BMP-3 Expression Vector

For high level expression of BMP-3 a  
 plasmid pALB3-782 was constructed. This plasmid is  
 identical to plasmid pALBP2-781, except that the BMP-2  
 20 gene (residues 2724-3133 of pALBP2-781) is replaced by a  
 gene encoding a form of mature BMP-3. The sequence of  
 this BMP-3 gene is:

ATGCGTAAAC AATGGATTGA ACCACGTAAC TGTGCTCGTC GTTATCTGAA  
AGTAGACTTT GCAGATATTG GCTGGAGTGA ATGGATTATC TCCCCCAAGT  
CCTTTGATGC CTATTATTGC TCTGGAGCAT GCCAGTTCCC CATGCCAAAG  
TCTTTGAAGC CATCAAATCA TGCTACCACATC CAGAGTATAAG TGAGAGCTGT  
5 GGGGGTCGTT CCTGGGATTC CTGAGCCTTG CTGTGTACCA GAAAAGATGT  
CCTCACTCAG TATTTTATTTC TTGATGAAA ATAAGAATGT AGTGCTTAAA  
GTATACCCCTA ACATGACAGT AGAGTCTTGC GCTTGCAGAT AACCTGGCAA  
AGAACTCATT TGAATGCTTA ATTCAAT

10 The host strain and growth conditions used for the production of BMP-3 were as described for BMP-2.

D. Expression of a BMP-2/7 Heterodimer in E. coli

15 Denatured and purified E. coli BMP-2 and BMP-7 monomers were isolated from E. coli inclusion body pellets by acidification and gel filtration as previously as previously described above. 125 ug of each BMP in 1% acetic acid were mixed and taken to dryness in a speed vac. The material was resuspended in 2.5 ml 50 mM Tris, 1.0 NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM glutathione (reduced), 1 mM glutathione (oxidized), pH 8.0. The sample was incubated at 23 C for one week.

20 The BMP-2/7 heterodimer was isolated by HPLC on a 25 x 0.46 cm Vydac C4 column. The sample was centrifuged in a microfuge for 5 minutes, and the supernatant was diluted with 22.5 ml 0.1% TFA.

25 A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

1.0 ml/minute

0-5'	20% B
5-10'	20-30% B
10-90'	30-50% B
5	90-100' 50-100% B

By SDS-PAGE analysis, the BMP-2/7 heterodimer eluted at about 23'.

Figure 10 is a comparison of the W-20 activity of E. coli BMP-2 and BMP-2/7 heterodimer, indicating greater 10 activity of the heterodimer.

F. Expression of BMP-2/3 Heterodimer in E. coli

BMP-2 and BMP-3 monomers were isolated as follows: to 1.0 g of frozen harvested cells expressing either BMP-2 or BMP-3 was added 3.3 ml of 100 mM Tris, 10 mM EDTA, pH 8.3. The cells were resuspended by vortexing vigorously. 33 ul of 100 mM PMSF in isopropanol was added and the cells lysed by one pass through a French pressure cell. The lysate was centrifuged in a microfuge 15 for 20 minutes at 4 C. The supernatant was discarded. The inclusion body pellet was taken up in 8.0 M quanidine hydrochloride, 0.25 M OTT, 0.5 M Tris, 5 mM EDTA, pH 8.5, 20 and heated at 37 C for one hour.

The reduced and denatured BMP monomers were isolated 25 by HPLC on a Supelco C4 guard column as follows:

A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

1.0 ml/minute

0-5' 1% B

5-40' 1-70% B

40-45' 70-100% B

5 Monomeric BMP eluted at 28-30'. Protein concentration was estimated by A280 and the appropriate extinction coefficient.

10 ug of BMP-2 and BMP-3 were combined and taken to dryness in a speed vac. To this was added 50 ul of 50 mM Tris, 1.0 M NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM reduced glutathione, 1 mM oxidized glutathione, pH 8.5. The sample was incubated at 23 for 3 days. The sample was analyzed by SDS-PAGE on a 16% tricine gel under reducing and nonreducing conditions. The BMP-2/3 heterodimer migrated at about 35 kd nonreduced, and reduced to BMP-2 monomer at about 13 kd and BMP-3 monomer at about 21 kd.

15 BMP-2/3 heterodimer produced in *E. coli* is tested for *in vivo* activity. (20 µg) at (ten days) is utilized to compare the *in vivo* activity of BMP-2/3 to BMP-2. BMP-2/3 implants showed no cartilage or bone forming activity, while the BMP-2 control implants showed the predicted amounts of bone and cartilage formation. The *in vivo* data obtained with BMP-2/3 is consistent with the *in vitro* data from the W-20 assay.

EXAMPLE 8 - W-20 BIOASSAYSA. Description of W-20 cells

Use of the W-20 bone marrow stromal cells as an indicator cell line is based upon the conversion of these cells to osteoblast-like cells after treatment with BMP-2 [R. S. Thies et al, "Bone Morphogenetic Protein alters W-20 stromal cell differentiation *in vitro*", Journal of Bone and Mineral Research, 5(2):305 (1990); and R. S. Thies et al, "Recombinant Human Bone Morphogenetic Protein 2 Induces Osteoblastic Differentiation in W-20-17 Stromal Cells", Endocrinology, in press (1992)]. Specifically, W-20 cells are a clonal bone marrow stromal cell line derived from adult mice by researchers in the laboratory of Dr. D. Nathan, Children's Hospital, Boston, MA. BMP-2 treatment of W-20 cells results in (1) increased alkaline phosphatase production, (2) induction of PTH stimulated cAMP, and (3) induction of osteocalcin synthesis by the cells. While (1) and (2) represent characteristics associated with the osteoblast phenotype, the ability to synthesize osteocalcin is a phenotypic property only displayed by mature osteoblasts. Furthermore, to date we have observed conversion of W-20 stromal cells to osteoblast-like cells only upon treatment with BMPs. In this manner, the in vitro activities displayed by BMP treated W-20 cells correlate with the in vivo bone forming activity known for BMPs.

Below two in vitro assays useful in comparison of BMP activities of novel osteoinductive molecules are described.

B. W-20 Alkaline Phosphatase Assay Protocol

5 W-20 cells are plated into 96 well tissue culture plates at a density of 10,000 cells per well in 200  $\mu$ l of media (DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 100 U/ml + 100  $\mu$ g/ml streptomycin. The cells are allowed to attach overnight 10 in a 95% air, 5% CO<sub>2</sub> incubator at 37°C.

The 200  $\mu$ l of media is removed from each well with a multichannel pipettor and replaced with an equal volume of test sample delivered in DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 1% penicillin-streptomycin. Test substances are assayed in triplicate.

15 The test samples and standards are allowed a 24 hour incubation period with the W-20 indicator cells. After the 24 hours, plates are removed from the 20 37°C incubator and the test media are removed from the cells.

The W-20 cell layers are washed 3 times with 200  $\mu$ l per well of calcium/magnesium free phosphate buffered saline and these washes are discarded.

25 50  $\mu$ l of glass distilled water is added to each well and the assay plates are then placed on a dry ice/ethanol bath for quick freezing. Once frozen, the

assay plates are removed from the dry ice/ethanol bath and thawed at 37°C. This step is repeated 2 more times for a total of 3 freeze-thaw procedures. Once complete, the membrane bound alkaline phosphatase is available for measurement.

5            50 µl of assay mix (50 mM glycine, 0.05% Triton X-100, 4 mM MgCl<sub>2</sub>, 5 mM p-nitrophenol phosphate, pH = 10.3) is added to each assay well and the assay plates are then incubated for 30 minutes at 37°C in a shaking waterbath at 60 oscillations per minute.

10            At the end of the 30 minute incubation, the reaction is stopped by adding 100 µl of 0.2 N NaOH to each well and placing the assay plates on ice.

15            The spectrophotometric absorbance for each well is read at a wavelength of 405 nanometers. These values are then compared to known standards to give an estimate of the alkaline phosphatase activity in each sample. For example, using known amounts of p-nitrophenol phosphate, absorbance values are generated.

20            This is shown in Table I.

---

Table I

Absorbance Values for Known Standards  
of P-Nitrophenol Phosphate

25            P-nitrophenol phosphate umoles      Mean absorbance (405 nm)

0.000	0
0.006	0.261 +/- .024
0.012	0.521 +/- .031
0.018	0.797 +/- .063

0.024	1.074 +/- .061
0.030	1.305 +/- .083

5 Absorbance values for known amounts of  
BMP-2 can be determined and converted to  $\mu$ moles of p-nitrophenol phosphate cleaved per unit time as shown in  
Table II.

Table II

10 Alkaline Phosphatase Values for W-20 Cells  
Treating with BMP-2

BMP-2 concentration ng/ml	Absorbance Reading 405 nmeters	umoles substrate per hour
0	0.645	0.024
1.56	0.696	0.026
3.12	0.765	0.029
6.25	0.923	0.036
12.50	1.121	0.044
25.0	1.457	0.058
50.0	1.662	0.067
100.0	1.977	0.080

15 These values are then used to compare the  
activities of known amounts of BMP heterodimers to BMP-2  
20 homodimer.

C. Osteocalcin RIA Protocol

25 W-20 cells are plated at  $10^6$  cells per well  
in 24 well multiwell tissue culture dishes in 2 mls of  
DME containing 10% heat inactivated fetal calf serum, 2  
mM glutamine. The cells are allowed to attach overnight  
30 in an atmosphere of 95% air 5% CO<sub>2</sub> at 37°C.

The next day the medium is changed to DME

containing 10% fetal calf serum, 2 mM glutamine and the test substance in a total volume of 2 ml. Each test substance is administered to triplicate wells. The test substances are incubated with the W-20 cells for a total of 96 hours with replacement at 48 hours by the same test medias.

At the end of 96 hours, 50  $\mu$ l of the test media is removed from each well and assayed for osteocalcin production using a radioimmunoassay for mouse osteocalcin. The details of the assay are described in the kit manufactured by Biomedical Technologies Inc., 378 Page Street, Stoughton, MA 02072. Reagents for the assay are found as product numbers BT-431 (mouse osteocalcin standard), BT-432 (Goat anti-mouse Osteocalcin), BT-431R (iodinated mouse osteocalcin), BT-415 (normal goat serum) and BT-414 (donkey anti goat IgG). The RIA for osteocalcin synthesized by W-20 cells in response to BMP treatment is carried out as described in the protocol provided by the manufacturer.

The values obtained for the test samples are compared to values for known standards of mouse osteocalcin and to the amount of osteocalcin produced by W-20 cells in response to challenge with known amounts of BMP-2. The values for BMP-2 induced osteocalcin synthesis by W-20 cells is shown in Table III.

Table III

## Osteocalcin Synthesis by W-20 Cells

	<u>BMP-2 Concentration ng/ml</u>	<u>Osteocalcin Synthesis ng/well</u>
5	0	0.8
	2	0.9
	4	0.8
	8	2.2
	16	2.7
	31	3.2
10	62	5.1
	125	6.5
	250	8.2
	500	9.4
	1000	10.0
15		

EXAMPLE 9 - ROSEN MODIFIED SAMPATH-REDDI ASSAY

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. USA, 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1% TFA, and the resulting solution added to 20 mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are

implanted subcutaneously in the abdominal thoracic area of 21-49 ay old male Long Evans rats. The implants are removed after 7-14 days. Half of each implant is used for alkaline phosphatase analysis [see, A. H. Reddi et al, Proc. Natl. Acad. Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 1  $\mu\text{m}$  glycolmethacrylate sections are stained with Von Kossa and acid fuschin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2, and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone.

The heterodimeric BMP proteins of this invention may be assessed for activity on this assay.

Numerous modifications and variations in practice of this invention are expected to occur to those skilled in the art. Such modifications and variations are encompassed within the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Israel, David  
Wolfman, Neil M.

(ii) TITLE OF INVENTION: Recombinant Bone Morphogenetic Protein  
Heterodimers, Compositions and Methods of Use.

(iii) NUMBER OF SEQUENCES: 30

## (iv) CORRESPONDENCE ADDRESS:

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Tape  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

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(B) REGISTRATION NUMBER: 32,245  
(C) REFERENCE/DOCKET NUMBER: GI-5192B

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## !) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1607 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 356..1543

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAAC TTG CAGGGAGAAT	60
AACTTGCAGCA CCCCCACTTG CGCCGGTGCC TTTGCCCGAG CGGAGCCTGC TTCGCCATCT	120
CCGAGCCCCA CCGCCCCCTCC ACTCCTCGGC CTTGCCCGAC ACTGAGACGC TGTTCCCAGC	180
GTGAAAAGAG AGACTGCGCG GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG	240
GACATTGGT CCTTGCAGCCA GGTCCCTTGA CCAGAGTTT TCCATGTGGA CGCTCTTCA	300
ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT CGACC ATG Met 1	358
 GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC CTC Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Pro Gln Val Leu 5 10 15	406
CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe 20 25 30	454
GCG GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val 35 40 45	502
CTG AGC GAG TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys Gln 50 55 60 65	550
AGA CCC ACC CCC AGC AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu Asp 70 75 80	598
CTG TAT CGC AGG CAC TCA GGT CAG CCG GGC TCA CCC GCC CCA GAC CAC Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp His 85 90 95	646
CGG TTG GAG AGG GCA GCC AGC CGA GCC AAC ACT GTG CGC AGC TTC CAC Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe His 100 105 110	694
CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG AGT GGG AAA ACA ACC His Glu Glu Ser Leu Glu Leu Pro Glu Thr Ser Gly Lys Thr Thr 115 120 125	742
CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG GAG TTT ATC Arg Arg Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe Ile 130 135 140 145	790
ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT TTA Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala Leu 150 155 160	838
GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile 165 170 175	886
AAA CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp	934

92

180	185	190	
ACC AGG TTG GTG AAT CAG AAT GCA AGC AGG TGG GAA ACT TTT GAT GTC Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Thr Phe Asp Val	195	200	982
205			
ACC CCC GCT GTG ATG CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His Gly	210	215	1030
220	225		
TTC GTG GTG GAA GTG GCC CAC TTG GAG GAG AAA CAA GGT GTC TCC AAG Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser Lys	230	235	1078
240			
AGA CAT GTT AGG ATA AGC AGG TCT TTG CAC CAA GAT GAA CAC AGC TGG Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser Trp	245	250	1126
255			
TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC CAT GAT GGA AAA GGG Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys Gly	260	265	1174
270			
CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC AAA CAG CGG His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg	275	280	1222
285			
AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC TTC Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe	290	295	1270
300	305		
AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His	310	315	1318
320			
GCC TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu	325	330	1366
335			
AAC TCC ACT AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn	340	345	1414
350			
TCT AAG ATT CCT AAG GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile	355	360	1462
365			
TCG ATG CTG TAC CTT GAC GAG AAT GAA AAG GTT GTA TTA AAG AAC TAT Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr	370	375	1510
380	385		
CAG GAC ATG GTT GTG GAG GGT TGT GGG TGT CGC TAGTACAGCA AAATTAAATA Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg	390	395	1563
CATAAAATATA TATATATATA TATATTTAG AAAAAAGAAA AAAA			1607

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 396 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Val	Ala	Gly	Thr	Arg	Cys	Leu	Leu	Ala	Leu	Leu	Leu	Pro	Gln	Val
1					5					10					15
Leu	Leu	Gly	Gly	Ala	Ala	Gly	Leu	Val	Pro	Glu	Leu	Gly	Arg	Arg	Lys
		20						25					30		
Phe	Ala	Ala	Ala	Ser	Ser	Gly	Arg	Pro	Ser	Ser	Gln	Pro	Ser	Asp	Glu
	35						40					45			
Val	Leu	Ser	Glu	Phe	Glu	Leu	Arg	Leu	Ser	Met	Phe	Gly	Leu	Lys	
	50					55				60					
Gln	Arg	Pro	Thr	Pro	Ser	Arg	Asp	Ala	Val	Val	Pro	Pro	Tyr	Met	Leu
	65					70				75				80	
Asp	Leu	Tyr	Arg	Arg	His	Ser	Gly	Gln	Pro	Gly	Ser	Pro	Ala	Pro	Asp
		85						90					95		
His	Arg	Leu	Glu	Arg	Ala	Ala	Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe
		100						105					110		
His	His	Glu	Glu	Ser	Leu	Glu	Glu	Leu	Pro	Glu	Thr	Ser	Gly	Lys	Thr
		115					120				125				
Thr	Arg	Arg	Phe	Phe	Asn	Leu	Ser	Ser	Ile	Pro	Thr	Glu	Glu	Phe	
	130					135				140					
Ile	Thr	Ser	Ala	Glu	Leu	Gln	Val	Phe	Arg	Glu	Gln	Met	Gln	Asp	Ala
	145					150				155				160	
Leu	Gly	Asn	Asn	Ser	Ser	Phe	His	His	Arg	Ile	Asn	Ile	Tyr	Glu	Ile
		165						170					175		
Ile	Lys	Pro	Ala	Thr	Ala	Asn	Ser	Lys	Phe	Pro	Val	Thr	Arg	Leu	Leu
		180						185				190			
Asp	Thr	Arg	Leu	Val	Asn	Gln	Asn	Ala	Ser	Arg	Trp	Glu	Thr	Phe	Asp
		195				200					205				
Val	Thr	Pro	Ala	Val	Met	Arg	Trp	Thr	Ala	Gln	Gly	His	Ala	Asn	His
		210				215					220				
Gly	Phe	Val	Val	Glu	Val	Ala	His	Leu	Glu	Glu	Lys	Gln	Gly	Val	Ser
	225					230				235				240	
Lys	Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu	His	Gln	Asp	Glu	His	Ser
		245						250				255			
Trp	Ser	Gln	Ile	Arg	Pro	Leu	Leu	Val	Thr	Phe	Gly	His	Asp	Gly	Lys
		260				265					270				
Gly	His	Pro	Leu	His	Lys	Arg	Glu	Lys	Arg	Gln	Ala	Lys	His	Gln	

275

280

285

Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp			
290	295	300	
Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr			
305	310	315	320
His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His			
325	330	335	
Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val			
340	345	350	
Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala			
355	360	365	
Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn			
370	375	380	
Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg			
385	390	395	

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1954 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 403..1626

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGGAGCGC GGAGCCCGGC CGGAAAGCTA	60		
GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG	120		
AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC	180		
ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG	240		
CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC	300		
GCACTGCTGC AGCTTCCCTG AGCCTTCCA GCAAGTTGT TCAAGATTGG CTGTCAAGAA	360		
TCATGGACTG TTATTATATG CCTTGTTTC TGTCAAGACA CC ATG ATT CCT GGT	414		
Met Ile Pro Gly			
1			
AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC	462		
Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly			
5	10	15	20

GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala 25 30 35	510
GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu 40 45 50	558
CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg 55 60 65	606
CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg 70 75 80	654
GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAA GAG CAG ATC CAC Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Gln Ile His 85 90 95 100	702
AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr 105 110 115	750
GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr 120 125 130	798
AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro 135 140 145	846
GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln 150 155 160	894
GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile 165 170 175 180	942
TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile 185 190 195	990
ACA CGA CTA CTG GAC ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr Arg Trp 200 205 210	1038
GAA ACT TTT GAT GTG AGC CCT GCG GTC CTT CGC TGG ACC CGG GAG AAG Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg Glu Lys 215 220 225	1086
CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr 230 235 240	1134
CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln 245 250 255 260	1162

GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC ACC TTT GGC Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val Thr Phe Gly 265 270 275	1230
CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG CGT His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys Arg 280 285 290	1278
AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAC TGC Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys 295 300 305	1326
CGG CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp 310 315 320	1374
TGG ATT GTG GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp 325 330 335 340	1422
TGC CCC TTT CCA CTG GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile 345 350 355	1470
GTG CAG ACC CTG GTC AAT TCT GTC AAT TCC AGT ATC CCC AAA GCC TGT Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys 360 365 370	1518
TGT GTG CCC ACT GAA CTG AGT GCC ATC TCC ATG CTG TAC CTG GAT GAG Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu 375 380 385	1566
TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG ATG GTA GTA GAG GGA Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly 390 395 400	1614
TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC Cys Gly Cys Arg 405	1666
CACACACACA CACCACATA ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC	1726
ACAGACTGCT TCCTTATAGC TGGACTTTA TTTAAAAAAA AAAAAAAA AATGGAAAAA	1786
ATCCCTAAC ATTACACCTTG ACCTTATTAG TGACTTTACG TGCAAATGTT TTGACCATAT	1846
TGATCATATA TTTTGACAAA ATATATTAT AACTACGTAT TAAAAGAAAA AAATAAAATG	1906
AGTCATTATT TTAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC	1954

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 408 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val  
 1 5 10 15

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys  
 20 25 30

Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly  
 35 40 45

\* Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met  
 50 55 60

Phe Gly Leu Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro  
 65 70 75 80

Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu  
 85 90 95

Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser  
 100 105 110

Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn  
 115 120 125

Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu  
 130 135 140

Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu  
 145 150 155 160

Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His  
 165 170 175

Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro  
 180 185 190

Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn  
 195 200 205

Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp  
 210 215 220

Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His  
 225 230 235 240

Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg  
 245 250 255

Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu  
 260 265 270

Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg  
 275 280 285

Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys  
 290 295 300

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val  
 305 310 315 320  
 Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr  
 325 330 335  
 Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 340 345 350  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile  
 355 360 365  
 Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu  
 370 375 380  
 Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met  
 385 390 395 400  
 Val Val Glu Gly Cys Gly Cys Arg  
 405

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1448 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 97..1389

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGACCGAGC GGCGCGGACG GCCGCCTGCC CCCTCTGCCA CCTGGGGCGG TGCGGGCCCG	60
GAGCCCGGAG CCCGGTAGC GCGTAGAGCC GGCGCG ATG CAC GTG CGC TCA CTG Met His Val Arg Ser Leu	114
1 5	
CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA CCC CTG TTC Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro Leu Phe	162
10 15 20	
CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC GAG GTG CAC Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu Val His	210
25 30 35	
TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG GAG ATG Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg Glu Met	258
40 45 50	
CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC CCG CGC CCG Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro	306
55 60 65 70	

CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu Asp Leu 75 80 85	354
TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Pro Gly Gly Gln Gly 90 95 100	402
TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu 105 110 115	450
GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val Met 120 125 130	498
AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg 135 140 145 150	546
TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC CCA GAA GGG Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu Gly 155 160 165	594
GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC TAC ATC CGG Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile Arg 170 175 180	642
GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT CAG GTG CTC Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr Gln Val Leu 185 190 195	690
CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC GAC AGC CGT Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser Arg 200 205 210	738
ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC ATC ACA GCC Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr Ala 215 220 225 230	786
ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG GGC CTG CAG Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu Gln 235 240 245	834
CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG GCG Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu Ala 250 255 260	882
GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC TTC ATG GTG Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met Val 265 270 275	930
GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC CGG TCC ACG Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile Arg Ser Thr 280 285 290	978
GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC AAG AAC CAG Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn Gln 295 300 305 310	1026

100

GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC AGC GAC CAG Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln 315 320 325	1074
AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC CGA GAC CTG Arg Gln Ala Cys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu 330 335 340	1122
GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC GCC TAC TAC Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr 345 350 355	1170
TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC ACC Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr 360 365 370	1218
AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC CCG GAA ACG Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr 375 380 385 390	1266
GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC ATC TCC GTC Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val 395 400 405	1314
CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA TAC AGA AAC Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn 410 415 420	1362
ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC GAGAATTCA Met Val Val Arg Ala Cys Gly Cys His 425 430	1409
ACCCTTTGGG GCCAAGTTT TCTGGATCCT CCATTGCTC	1448

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 431 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro

65	70	101	75	80
Met	Phe	Met	Leu	Asp
			Leu	Tyr
			Asn	Ala
			Met	Ala
			Val	Glu
			Glu	Gly
				Gly
			85	90
				95
Gly	Pro	Gly	Gly	Gln
			Gly	Gly
			Phe	Ser
			Tyr	Pro
			Tyr	Lys
			Ala	Val
			Phe	Ser
			100	105
				110
Thr	Gln	Gly	Pro	Pro
			Leu	Ala
			Ser	Leu
			Gln	Asp
			Ser	Ser
			His	Phe
			Leu	Thr
			115	120
				125
Asp	Ala	Asp	Met	Val
			Met	Ser
			Phe	Val
			Asn	Leu
			Val	Glu
				His
				Asp
				Lys
			130	135
				140
Glu	Phe	Phe	His	Pro
			Arg	Tyr
			His	His
			Arg	Glu
			Phe	Phe
			Arg	Asp
				Leu
			145	150
				155
				160
Ser	Lys	Ile	Pro	Glu
			Gly	Glu
			Ala	Val
			Thr	Ala
			Ala	Glu
			Phe	Arg
			Ile	
			165	170
				175
Tyr	Lys	Asp	Tyr	Ile
			Arg	Arg
			Glu	Phe
			Asp	Asn
			Glu	Thr
			Thr	Phe
			Phe	Arg
			Ile	
			180	185
				190
Ser	Val	Tyr	Gln	Val
			Leu	Gln
			Glu	His
			Leu	Gly
			Gly	Arg
			Glu	Ser
			Ser	Asp
			Asp	Leu
				195
				200
				205
Phe	Leu	Leu	Asp	Ser
			Arg	Thr
			Leu	Trp
			Ala	Ser
			Glu	Glu
			Gly	Trp
			Trp	Leu
				210
				215
				220
Val	Phe	Asp	Ile	Thr
			Ala	Thr
			Ser	Asn
			His	Trp
			Trp	Val
			Val	Val
			Asn	Pro
				Arg
			225	230
				235
				240
His	Asn	Leu	Gly	Leu
			Gln	Gln
			Leu	Leu
			Ser	Val
			Glu	Glu
			Thr	Leu
			Asp	Gly
			Gly	Gln
				245
				250
				255
Ile	Asn	Pro	Lys	Leu
			Ala	Gly
			Leu	Ile
			Gly	Gly
			Arg	His
			Gly	Pro
			Asn	Gln
				Asn
			260	265
				270
Lys	Gln	Pro	Phe	Met
			Val	Val
			Ala	Phe
			Phe	Phe
			Lys	Ala
			Ala	Thr
			Gly	Glu
				275
				280
				285
Arg	Ser	Ile	Arg	Ser
			Thr	Gly
			Ser	Lys
			Gln	Arg
			Arg	Ser
			Gln	Gln
			Asn	Asn
				Arg
			290	295
				300
Lys	Thr	Pro	Lys	Asn
			Gln	Glu
			Ala	Leu
			Glu	Arg
			Met	Met
			Ala	Asn
			Asn	Val
			Ala	Glu
				305
				310
				315
				320
Asn	Ser	Ser	Ser	Asp
			Gln	Gln
			Arg	Ala
			Cys	Cys
			Lys	Lys
			His	His
			Glu	Glu
			Leu	Tyr
				325
				330
				335
Val	Ser	Phe	Arg	Asp
			Leu	Gly
			Trp	Gln
			Asp	Asp
			Trp	Ile
			Ile	Ile
			Ala	Pro
			Glu	Glu
				340
				345
				350
Gly	Tyr	Ala	Ala	Tyr
			Tyr	Tyr
			Cys	Glu
			Glu	Gly
			Cys	Glu
			Ala	Phe
			Phe	Pro
			Leu	Leu
			Asn	Asn
				355
				360
				365
Ser	Tyr	Met	Asn	Ala
			Asn	Thr
			His	Ala
			Ile	Val
			Gln	Gln
				370
				375
				380
Phe	Ile	Asn	Pro	Glu
			Thr	Val
			Pro	Lys
			Pro	Cys
			Cys	Cys
			Ala	Pro
			Thr	Gln

385	390	395	400
Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile			
405 410 415			
Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His			
420 425 430			

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2923 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: Human placenta

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Stratagene catalog #936203 Human placenta  
cDNA library
- (B) CLONE: BMP6C35

(viii) POSITION IN GENOME:

- (C) UNITS: bp

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 160..1701

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 1282..1698

(ix) FEATURE:

- (A) NAME/KEY: mRNA
- (B) LOCATION: 1..2923

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC GAGAGGTGGC	60
GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG GCCTCGCTCC GCCGCTCCAC	120
GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC CGGGCGGGG ATG CCG GGG CTG GGG Met Pro Gly Leu Gly	174
-374 -370	=
CGG AGG GCG CAG TGG CTG TGC TGG TGG GGG CTG CTG TGC AGC TGC Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly Leu Leu Cys Ser Cys	222
-365 -360 -355	=
TGC GGG CCC CCG CCG CTG CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC	270

Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala  
 -350 -345 -340

GCC GCC GGG GGG CAG CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG  
 Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Ser Pro Gly Arg Thr  
 -335 -330 -325

GAG CAG CCG CCG CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG  
 Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Gly Phe Leu Tyr Arg  
 -320 -315 -310

CGG CTC AAG ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG  
 Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln Lys Glu Ile Leu Ser  
 -305 -300 -295 -290

GTG CTG GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG  
 Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln  
 -285 -280 -275

CCG CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG  
 Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Gln Gln Gln Gln Gln  
 -270 -265 -260

CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG CCC  
 Gln Leu Pro Arg Gly Glu Pro Pro Gly Arg Leu Lys Ser Ala Pro  
 -255 -250 -245

CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC GAC GAG  
 Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn Asp Glu  
 -240 -235 -230

GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC CAC GAA GCA  
 Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro His Glu Ala  
 -225 -220 -215 -210

GCC AGC TCG TCC CAG CGT CGG CAG CCC CCG GGC GCC GCG CAC CCG  
 Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Gly Ala Ala His Pro  
 -205 -200 -195

CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT GGC AGC GGC GGC GCG  
 Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser Gly Ser Gly Gly Ala  
 -190 -185 -180

TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC TTC CTC AAC GAC GCG GAC  
 Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp  
 -175 -170 -165

ATG GTC ATG AGC TTT GTG AAC CTG GTG GAG TAC GAC AAG GAG TTC TCC  
 Met Val Met Ser Phe Val Asn Leu Val Glu Tyr Asp Lys Glu Phe Ser  
 -160 -155 -150

CCT CGT CAG CGA CAC CAC AAA GAG TTC AAG TTC AAC TTA TCC CAG ATT  
 Pro Arg Gln Arg His His Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile  
 -145 -140 -135 -130

CCT GAG GGT GAG GTG GTG ACG GCT GCA GAA TTC CGC ATC TAC AAG GAC  
 Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp  
 -125 -120 -115

TGT GTT ATG GGG AGT TTT AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT

942

366

414

462

510

558

606

654

702

750

798

846

894

990

Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr			
-110	-105	-100	
CAA GTC TTA CAG GAG CAT CAG CAC AGA GAC TCT GAC CTG TTT TTG TTG			1038
Gln Val Leu Gln Glu His Gln His Arg Asp Ser Asp Leu Phe Leu Leu			
-95	-90	-85	
GAC ACC CGT GTA GTA TGG GCC TCA GAA GAA GGC TGG CTG GAA TTT GAC			1086
Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp			
-80	-75	-70	
ATC ACG GCC ACT AGC AAT CTG TGG GTT GTG ACT CCA CAG CAT AAC ATG			1134
Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr Pro Gln His Asn Met			
-65	-60	-55	-50
GGG CTT CAG CTG AGC GTG GTG ACA AGG GAT GGA GTC CAC GTC CAC CCC			1182
Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly Val His Val His Pro			
-45	-40	-35	
CGA GCC GCA GGC CTG GTG GGC AGA GAC GGC CCT TAC GAT AAG CAG CCC			1230
Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro			
-30	-25	-20	
TTC ATG GTG GCT TTC TTC AAA GTG AGT GAG GTC CAC GTG CGC ACC ACC			1278
Phe Met Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg Thr Thr			
-15	-10	-5	
AGG TCA GCC TCC AGC CGG CGC CGA CAA CAG AGT CGT AAT CGC TCT ACC			1326
Arg Ser Ala Ser Ser Arg Arg Gln Gln Ser Arg Asn Arg Ser Thr			
1	5	10	15
CAG TCC CAG GAC GTG GCG CGG GTC TCC AGT GCT TCA GAT TAC AAC AGC			1374
Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala Ser Asp Tyr Asn Ser			
20	25	30	
AGT GAA TTG AAA ACA GGC TGC AGG AAG CAT GAG CTG TAT GTG AGT TTC			1422
Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu Leu Tyr Val Ser Phe			
35	40	45	
CAA GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA CCC AAG GGC TAT GCT			1470
Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala			
50	55	60	
GCC AAT TAC TGT GAT GGA GAA TGC TCC TTC CCA CTC AAC GCA CAC ATG			1518
Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met			
65	70	75	
AAT GCA ACC AAC CAC GCG ATT GTG CAG ACC TTG GTT CAC CTT ATG AAC			1566
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Leu Met Asn			
80	85	90	95
CCC GAG TAT GTC CCC AAA CCG TGC TGT GCG CCA ACT AAG CTA AAT GCC			1614
Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala			
100	105	110	
ATC TCG GTT CTT TAC TTT GAT GAC AAC TCC AAT GTC ATT CTG AAA AAA			1662
Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu Lys Lys			
115	120	125	
TAC AGG AAT ATG GTT GTA AGA GCT TGT GGA TGC CAC TAACTCGAAA			1708

Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 130 135 140

CCAGATGCTG	GGGACACACA	TTCTGCCTTG	GATTCCCTAGA	TTACATCTGC	CTTAAAAAAA	1768
CACGGAAGCA	CAGTTGGAGG	TGGGACGATG	AGACTTTGAA	ACTATCTCAT	GCCAGTGCCT	1828
TATTACCCAG	GAAGATTATA	AAGGACCTCA	TTAATAATTT	GCTCACTGG	TAAATGACGT	1888
GAGTAGTTGT	TGGTCTGTAG	CAAGCTGAGT	TTGGATGTCT	GTAGCATAAG	GTCTGGTAAC	1948
TGCAGAAACA	TAACCGTGAA	GCTCTTCCTA	CCCTCCTCCC	CCAAAAACCC	ACCAAAATTA	2008
GTTTTAGCTG	TAGATCAAGC	TATTTGGGGT	GTTTGTAGT	AAATAGGGAA	AATAATCTCA	2068
AAGGAGTTAA	ATGTATTCTT	GGCTAAAGGA	TCAGCTGGTT	CAGTACTGTC	TATCAAAGGT	2128
AGATTTACA	GAGAACAGAA	ATCGGGGAAG	TGGGGGAAAC	GCCTCTGTT	AGTCATTCC	2188
CAGAAGTCCA	CAGGACGCCAC	AGCCCAGGCC	ACAGCCAGGG	CTCCACGGGG	CGCCCTTGTC	2248
TCAGTCATTG	CTGTTGTATG	TCCTGCTGG	AGTTTGTG	GTGTGAAAAT	ACACTTATT	2308
CAGCCAAAC	ATACCATTTC	TACACCTCAA	TCCTCCATT	GCTGTACTCT	TTGCTAGTAC	2368
CAAAAGTAGA	CTGATTACAC	TGAGGTGAGG	CTACAAGGGG	TGTGTAACCG	TGTAACACGT	2428
GAAGGCAGTG	CTCACCTCTT	CTTACCCAGA	ACGGTTCTT	GACCAGCACA	TTAACTTCTG	2488
GAATGCCGGC	TCTAGTACCT	TTTCAGTAAA	GTGGTTCTCT	GCCTTTTAC	TATACAGCAT	2548
ACCACGCCAC	AGGGTTAGAA	CCAACGAAGA	AAATAAAATG	AGGGTGCCCA	GCTTATAAGA	2608
ATGGTGTAG	GGGGATGAGC	ATGCTGTTA	TGAACGGAAA	TCATGATTTC	CCTGTAGAAA	2668
GTGAGGCTCA	GATTAAATTT	TAGAATATTT	TCTAAATGTC	TTTTTCACAA	TCATGTGACT	2728
GGGAAGGCAA	TTTCATACTA	AACTGATTAA	ATAATACATT	TATAATCTAC	AACTGTTTGC	2788
ACTTACAGCT	TTTTTGTA	ATATAAACTA	TAATTTATTG	TCTATTTAT	ATCTGTTTG	2848
CTGTGGCGTT	GGGGGGGGGG	CCGGGCTTT	GGGGGGGGGG	GTTTGTGTTGG	GGGGTGTGCGT	2908
GGTGTGGCG	GGCGG					2923

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 513 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp	Gly		
-374	-370	-365	-360

Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro  
 -355 -350 -345

Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly  
 -340 -335 -330

Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser  
 -325 -320 -315

Gly Phe Leu Tyr Arg Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln  
 -310 -305 -300 -295

Lys Glu Ile Leu Ser Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu  
 -290 -285 -280

His Gly Leu Gln Gln Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu  
 -275 -270 -265

Gln Gln Gln Gln Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg  
 -260 -255 -250

Leu Lys Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser  
 -245 -240 -235

Ala Asp Asn Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser  
 -230 -225 -220 -215

Trp Pro His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro  
 -210 -205 -200

Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser  
 -195 -190 -185

Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe  
 -180 -175 -170

Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu Tyr  
 -165 -160 -155

Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu Phe Lys Phe  
 -150 -145 -140 -135

Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe  
 -130 -125 -120

Arg Ile Tyr Lys Asp Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe  
 -115 -110 -105

Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu His Gln His Arg Asp Ser  
 -100 -95 -90

Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly  
 -85 -80 -75

Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr  
 -70 -65 -60 -55

Pro Gln His Asn Met Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly  
 -50 -45 -40

Val His Val His Pro Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro  
                   -35                          -30                          -25  
  
 Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe Lys Val Ser Glu Val  
                   -20                          -15                          -10  
  
 His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser  
       -5                                  1                          5                          10  
  
 Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala  
       15                                  20                          25  
  
 Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu  
       30                                  35                          40  
  
 Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala  
       45                                  50                          55  
  
 Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro  
       60                                  65                          70  
  
 Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu  
       75                                  80                          85                          90  
  
 Val His Leu Met Asn Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro  
       95                                  100                          105  
  
 Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn  
       110                                  115                          120  
  
 Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys  
       125                                  130                          135  
  
 His

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2153 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens
  - (H) CELL LINE: U2-OS osteosarcoma
- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: U2-OS human osteosarcoma cDNA library
  - (B) CLONE: U2-16
- (viii) POSITION IN GENOME:
  - (C) UNITS: bp
- (ix) FEATURE:
  - (A) NAME/KEY: CDS

(B) LOCATION: 699..2063

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 1647..2060

## (ix) FEATURE:

- (A) NAME/KEY: mRNA  
 (B) LOCATION: 1..2153

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA GGGATTGAAT	60
GGACTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC ATTACTTGA ATAGTACAAC	120
CTAGAGTATT ATTTTACACT AAGACGACAC AAAAGATGTT AAAGTTATCA CCAAGCTGCC	180
GGACAGATAT ATATTCCAAC ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC	240
AGGATTGTT TTGGAAAGAG CTCAAGGGTT GAGAAGAACT CAAAAGCAAG TGAAGATTAC	300
TTTGGGAACT ACAGTTTATC AGAACATCAA CTTTGCTAA TTCAAATACC AAAGGCCTGA	360
TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC AAATAATATT AGCCGTCTTC	420
TGCTACATCA ATGCAGCAAA AACTCTAAC AACTGTGGAT AATTGGAAAT CTGAGTTTCA	480
GCTTTCTTAG AAATAACTAC TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAAA	540
TCGGTGAGGA TGTTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT	600
TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTTT AAGAGGACAA	660
GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAAA ATG CAT CTG ACT GTA Met His Leu Thr Val -316-315	713
 TTT TTA CTT AAG GGT ATT GTG GGT TTC CTC TGG AGC TGC TGG GTT CTA Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp Ser Cys Trp Val Leu -310                    -305                    -300	761
 TG TG GGT TAT GCA AAA GGA GGT TTG GGA GAC AAT CAT GTT CAC TCC AGT Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn His Val His Ser Ser -295                    -290                    -285                    -280	809
 TTT ATT TAT AGA AGA CTA CGG AAC CAC GAA AGA CGG GAA ATA CAA AGG Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg Arg Glu Ile Gln Arg -275                    -270                    -265	857
 GAA ATT CTC TCT ATC TTG GGT TTG CCT CAC AGA CCC AGA CCA TTT TCA Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro Phe Ser -260                    -255                    -250	905
 CCT GGA AAA ATG ACC AAT CAA GCG TCC TCT GCA CCT CTC TTT ATG CTG Pro Gly Lys Met Thr Asn Gln Ala Ser Ser Ala Pro Leu Phe Met Leu -245                    -240                    -235	953
 GAT CTC TAC AAT GCC GAA GAA AAT CCT GAA GAG TCG GAG TAC TCA GTA	1001

Asp Leu Tyr Asn Ala Glu Glu Asn Pro Glu Glu Ser Glu Tyr Ser Val -230 -225 -220		
AGG GCA TCC TTG GCA GAA GAG ACC AGA GGG GCA AGA AAG GGA TAC CCA Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala Arg Lys Gly Tyr Pro -215 -210 -205 -200		1049
GCC TCT CCC AAT GGG TAT CCT CGT CGC ATA CAG TTA TCT CGG ACG ACT Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile Gln Leu Ser Arg Thr Thr -195 -190 -185		1097
CCT CTG ACC ACC CAG AGT CCT CTC CTA GCC AGC CTC CAT GAT ACC AAC Pro Leu Thr Thr Gln Ser Pro Pro Leu Ala Ser Leu His Asp Thr Asn -180 -175 -170		1145
TTT CTG AAT GAT GCT GAC ATG GTC ATG AGC TTT GTC AAC TTA GTT GAA Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu -165 -160 -155		1193
AGA GAC AAG GAT TTT TCT CAC CAG CGA AGG CAT TAC AAA GAA TTT CGA Arg Asp Lys Asp Phe Ser His Gln Arg Arg His Tyr Lys Glu Phe Arg -150 -145 -140		1241
TTT GAT CTT ACC CAA ATT CCT CAT GGA GAG GCA GTG ACA GCA GCT GAA Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala Val Thr Ala Ala Glu -135 -130 -125 -120		1289
TTC CGG ATA TAC AAG GAC CGG AGC AAC CGA TTT GAA AAT GAA ACA Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg Phe Glu Asn Glu Thr -115 -110 -105		1337
ATT AAG ATT AGC ATA TAT CAA ATC ATC AAG GAA TAC ACA AAT AGG GAT Ile Lys Ile Ser Ile Tyr Gln Ile Lys Glu Tyr Thr Asn Arg Asp -100 -95 -90		1385
GCA GAT CTG TTC TTG TTA GAC ACA AGA AAG GCC CAA GCT TTA GAT GTG Ala Asp Leu Phe Leu Asp Thr Arg Lys Ala Gln Ala Leu Asp Val -85 -80 -75		1433
GGT TGG CTT GTC TTT GAT ATC ACT GTG ACC AGC AAT CAT TGG GTG ATT Gly Trp Leu Val Phe Asp Ile Thr Val Thr Ser Asn His Trp Val Ile -70 -65 -60		1481
AAT CCC CAG AAT AAT TTG GGC TTA CAG CTC TGT GCA GAA ACA GGG GAT Asn Pro Gln Asn Asn Leu Gly Leu Gln Leu Cys Ala Glu Thr Gly Asp -55 -50 -45 -40		1529
GGA CGC AGT ATC AAC GTA AAA TCT GCT GGT CTT GTG GGA AGA CAG GGA Gly Arg Ser Ile Asn Val Lys Ser Ala Gly Leu Val Gly Arg Gln Gly -35 -30 -25		1577
CCT CAG TCA AAA CAA CCA TTC ATG GTG GCC TTC TTC AAG GCG AGT GAG Pro Gln Ser Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Ser Glu -20 -15 -10		1625
GTA CTT CTT CGA TCC GTG AGA GCA GGC AAC AAA CGA AAA AAT CAA AAC Val Leu Leu Arg Ser Val Arg Ala Ala Asn Lys Arg Lys Asn Gln Asn -5 1 5		1673
CGC AAT AAA TCC AGC TCT CAT CAG GAC TCC TCC AGA ATG TCC AGT GTT		1721

110

Arg Asn Lys Ser Ser Ser His Gln Asp Ser Ser Arg Met Ser Ser Val	10	15	20	25	
GGA GAT TAT AAC ACA AGT GAG CAA AAA CAA GCC TGT AAG AAG CAC GAA					1769
Gly Asp Tyr Asn Thr Ser Glu Gln Lys Ala Cys Lys His Glu	30	35		40	
CTC TAT GTG AGC TTC CGG GAT CTG GGA TGG CAG GAC TGG ATT ATA GCA					1817.
Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala	45	50	55		
CCA GAA GGA TAC GCT GCA TTT TAT TGT GAT GGA GAA TGT TCT TTT CCA					1865
Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly Glu Cys Ser Phe Pro	60	65	70		
CTT AAC GCC CAT ATG AAT GCC ACC AAC CAC GCT ATA GTT CAG ACT CTG					1913
Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu	75	80	85		
GTT CAT CTG ATG TTT CCT GAC CAC GTA CCA AAG CCT TGT TGT GCT CCA					1961
Val His Leu Met Phe Pro Asp His Val Pro Lys Pro Cys Cys Ala Pro	90	95	100	105	
ACC AAA TTA AAT GCC ATC TCT GTT CTG TAC TTT GAT GAC AGC TCC AAT					2009
Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn	110	115		120	
GTC ATT TTG AAA AAA TAT AGA AAT ATG GTA GTA CGC TCA TGT GGC TGC					2057
Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ser Cys Gly Cys	125	130	135		
CAC TAATATTAAA TAATATTGAT AATAACAAAA AGATCTGTAT TAAGGTTTAT					2110
His .					
GGCTGCAATA AAAAGCATAAC TTTCAGACAA ACAGAAAAAA AAA					2153

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 454 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp  
 -316 -315 -310 -305

Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn  
 -300 -295 -290 -285

His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg  
 -280 -275 -270

Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg  
 -265 -260 -255

Pro Arg Pro Phe Ser Pro Gly Lys Met Thr Asn Gln Ala Ser Ser Ala  
 -250 -245 -240

Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Glu Glu Asn Pro Glu Glu  
 -235 -230 -225

Ser Glu Tyr Ser Val Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala  
 -220 -215 -210 -205

Arg Lys Gly Tyr Pro Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile Gln  
 -200 -195 -190

Leu Ser Arg Thr Thr Pro Leu Thr Thr Gln Ser Pro Pro Leu Ala Ser  
 -185 -180 -175

Leu His Asp Thr Asn Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe  
 -170 -165 -160

Val Asn Leu Val Glu Arg Asp Lys Asp Phe Ser His Gln Arg Arg His  
 -155 -150 -145

Tyr Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala  
 -140 -135 -130 -125

Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg  
 -120 -115 -110

Phe Glu Asn Glu Thr Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu  
 -105 -100 -95

Tyr Thr Asn Arg Asp Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala  
 -90 -85 -80

Gln Ala Leu Asp Val Gly Trp Leu Val Phe Asp Ile Thr Val Thr Ser  
 -75 -70 -65

Asn His Trp Val Ile Asn Pro Gln Asn Asn Leu Gly Leu Gln Leu Cys  
 -60 -55 -50 -45

Ala Glu Thr Gly Asp Gly Arg Ser Ile Asn Val Lys Ser Ala Gly Leu  
 -40 -35 -30

Val Gly Arg Gln Gly Pro Gln Ser Lys Gln Pro Phe Met Val Ala Phe  
 -25 -20 -15

Phe Lys Ala Ser Glu Val Leu Leu Arg Ser Val Arg Ala Ala Asn Lys  
 -10 -5 1

Arg Lys Asn Gln Asn Arg Asn Lys Ser Ser Ser His Gln Asp Ser Ser  
 5 10 15 20

Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln Ala  
 25 30 35

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
 40 45 50

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly  
 55 60 65

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 70 75 80  
 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys  
 85 90 95 100  
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 105 110 115  
 Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
 120 125 130  
 Arg Ser Cys Gly Cys His  
 135

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1003 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens
  - (F) TISSUE TYPE: Human Heart
- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: Human heart cDNA library stratagene catalog #936208
  - (B) CLONE: hH38
- (viii) POSITION IN GENOME:
  - (C) UNITS: bp
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 8..850
- (ix) FEATURE:
  - (A) NAME/KEY: mat\_peptide
  - (B) LOCATION: 427..843
- (ix) FEATURE:
  - (A) NAME/KEY: mRNA
  - (B) LOCATION: 1..997
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC  
 Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile  
 -139 -135 -130

CCG GCT GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val -125 -120 -115 -110	9
CCC AGC ATC CAC CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG Pro Ser Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln -105 -100 -95	14:
GTG GTC CAG GAG CAG TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT Val Val Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp -90 -85 -80	19:
CTT CAG ACG CTC CGA GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC Leu Gln Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val -75 -70 -65	24:
ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA Thr Ala Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly -60 -55 -50	28:
CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC GTG GAT CCT GGC Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly -45 -40 -35 -30	33:
CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe -25 -20 -15	38:
GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg -10 -5 1	43:
GCA GTG AGG CCA CTG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG Ala Val Arg Pro Leu Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu 5 10 15	48:
CCG CAG GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser 20 25 30 35	52:
CAC GGC CGG CAG GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln 40 45 50	57:
GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala 55 60 65	62:
TAT TAC TGT GAG GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn 70 75 80	67:
GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro 85 90 95	72:
AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr 100 105 110 115	76:

TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His 120 125 130	817
CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCC CGCCCAGCCC Arg Asn Met Val Val Lys Ala Cys Gly Cys His 135 140	870
TACTGCAGCC ACCCTTCTCA TCTGGATCGG GCCCTGCAGA GGCAAGAAAAC CCTTAAATGC TGTCACAGCT CAAGCAGGAG TGTCAGGGGC CCTCACTCTC GGTGCCTACT TCCTGTCAGG CTTCTGGGAA TTC	930 990 1003

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 281 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala  
 -139 -135 -130 -125

Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser  
 -120 -115 -110

Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val  
 -105 -100 -95

Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln  
 -90 -85 -80

Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala  
 -75 -70 -65 -60

Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg  
 -55 -50 -45

Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala  
 -40 -35 -30

Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val  
 -25 -20 -15

Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val  
 -10 -5 1 5

Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln  
 10 15 20

Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly  
 25 30 35

Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu

40

45

50

Gly	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr
55										60				65	
Cys	Glu	Gly	Glu	Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr
70						75						80			85
Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala
90										95				100	
Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	Ser	Val
105										110				115	
Leu	Tyr	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg	Lys	His	Arg	Asn
120									125				130		
Met	Val	Val	Lys	Ala	Cys	Gly	Cys	His							
135						140									

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3623 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pALBP2-781

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2724..3071

(ix) FEATURE:

- (A) NAME/KEY: terminator
- (B) LOCATION: 3150..3218

(ix) FEATURE:

- (A) NAME/KEY: RBS
- (B) LOCATION: 2222..2723

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGAAAGGG CCTCGTGATA CGCCTATTAA TATAGGTTAA TGTCAATGATA ATAATGGTTT	60
CTTAGACGTC AGGTGGCACT TTTGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTAA	120
TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT	180
AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTT	240
TTGCGGCATT TTGCCTTCCT GTTTTGCTC ACCCAGAAC GCTGGTGAAA GTAAAAGATG	300
CTGAAGATCA GTTGGGTGCA CGAGTGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA	360

TCCCTTGAGAG	TTTCGCCCCC	GAAGAACGTT	TTCCAATGAT	GAGCACTTT	AAAGTTCTGC	420
TATGTGGCGC	GGTATTATCC	CGTATTGACG	CCGGGCAAGA	GCAACTCGGT	CGCCGCATAC	480
ACTATTCTCA	GAATGACTTG	GTTGAGTACT	CACCAGTCAC	AGAAAAGCAT	CTTACGGATG	540
GCATGACAGT	AAGAGAATTA	TGCAGTGCTG	CCATAACCAT	GAGTGATAAC	ACTGCGGCCA	600
ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTG	CACAACATGG	660
GGGATCATGT	AACTCGCCTT	GATCGTTGGG	AACCGGAGCT	GAATGAAGCC	ATACCAAACG	720
ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	TGGCAACAAC	GTTGCGAAA	CTATTAAC TG	780
GCGAACTACT	TACTCTAGCT	TCCC GGCAAC	AATTAATAGA	CTGGATGGAG	GCGGATAAAG	840
TTGCAGGACC	ACTTCTGCGC	TCGGCCCTTC	CGGCTGGCTG	GTTTATTGCT	GATAAATCTG	900
GAGCCGGTGA	CGGTGGGTCT	CGCGGTATCA	TTGCAGCACT	GGGGCCAGAT	GGTAAGCCCT	960
CCCGTATCGT	AGTTATCTAC	ACGACGGGGA	GTCAGGCAAC	TATGGATGAA	CGAAATAGAC	1020
AGATCGCTGA	GATAGGTGCC	TCACTGATTA	AGCATTGGTA	ACTGTCAGAC	CAAGTTACT	1080
CATATATACT	TTAGATTGAT	TTAAAACCTTC	ATTTTTAATT	AAAAAGGATC	TAGGTGAAGA	1140
TCCTTTTG	TAATCTCATG	ACCAAAATCC	CTTAACGTGA	GTTTCGTT	CACTGAGCGT	1200
CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CTTGAGATCC	TTTTTTCTG	CGCGTAATCT	1260
GCTGCTTGCA	AACAAAAAAA	CCACCGCTAC	CAGCGGTGGT	TTGTTGCCG	GATCAAGAGC	1320
TACCAACTCT	TTTCCGAAG	GTAACTGGCT	TCAGCAGAGC	GCAGATACCA	AATACTGTCC	1380
TTCTAGTGT	GCCGTAGTTA	GGCCACCACT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	1440
TCGCTCTGCT	AATCCTGTTA	CCAGTGGCTG	CTGCCAGTGG	CGATAAGTCG	TGTCTTACCG	1500
GGTTGGACTC	AAGACGATAG	TTACCGGATA	AGGCGCAGCG	GTCGGGCTGA	ACGGGGGGTT	1560
CGTGCACACA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	CTACAGCGTG	1620
AGCATTGAGA	AAGGCCACG	CTTCCCGAAG	GGAGAAAGGC	GGACAGGTAT	CCGGTAAGCG	1680
GCAGGGTCGG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG	GGAAACGCC	TGGTATCTTT	1740
ATAGTCCTGT	CGGGTTTCGC	CACCTCTGAC	TTGAGCGTCG	ATTTTGATGA	TGCTCGTCAG	1800
GGGGCGGAG	CCTATGGAAA	AACGCCAGCA	ACCGGGCCTT	TTTACGGTTC	CTGGCCTTT	1860
GCTGGCCTT	TGCTCACATG	TTCTTCCTG	CGTTATCCCC	TGATTCTGTG	GATAACCGTA	1920
TTACCGCCTT	TGAGTGAGCT	GATAACCGCTC	GCCGCAGCCG	AACGACCGAG	CGCAGCGAGT	1980
CAGTGAGCGA	GGAAGCGGAA	GAGCGCCCAA	TACGCAAACC	GCCTCTCCCC	GCGCGTTGGC	2040
CGATTCAATTA	ATGCAGAATT	GATCTCTCAC	CTACCAAACA	ATGCCCTT	GCAAAAAATA	2100
AATTCAATATA	AAAAACATAC	AGATAACCAT	CTGCGGTGAT	AAATTATCTC	TGGCGGTGTT	2160

GACATAAAATA	CCACTGGCGG	TGATACTGAG	CACATCAGCA	GGACGCACTG	ACCACCATGA	2220
AGGTGACGCT	CTTAAAATT	AAGCCCTGAA	GAAGGGCAGC	ATTCAAAGCA	GAAGGGTTTG	2280
GGGTGTGTGA	TACGAAACGA	AGCATTGGCC	GTAAGTGCAG	TTCCGGATT	GCTGCCAATG	2340
TGCCAATCGC	GGGGGGTTTT	CGTTCAGGAC	TACAAC TGCC	ACACACCACC	AAAGCTAACT	2400
GACAGGAGAA	TCCAGATGGA	TGCACAAACA	CGCCGCCGCG	AACGTCGCGC	AGAGAACAG	2460
GCTCAATGGA	AAGCAGCAA	TCCCCTGTTG	GTTGGGGTAA	GCGAAAACC	AGTTCCGAAA	2520
GATTTTTTA	ACTATAAACG	CTGATGGAAG	CGTTTATGCG	GAAGAGGTAA	AGCCCTTCCC	2580
GAGTAACAAA	AAAACAACAG	CATAAATAAC	CCCGCTCTTA	CACATTCCAG	CCCTGAAAAA	2640
GGGCATCAAA	TTAAACCACA	CCTATGGTGT	ATGCATTAT	TTGCATACAT	TCAATCAATT	2700
GTTATCTAAG	GAAATACTTA	CAT ATG CAA GCT AAA CAT AAA CAA CGT AAA				2750
	Met Gln Ala Lys His Lys Gln Arg Lys					
	1			5		
CGT CTG AAA TCT AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC TTC AGT						2798
Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser						
10	15		20		25	
GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC						2846
Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala						
30	35		40			
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC						2894
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn						
45	50		55			
TCC ACT AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT						2942
Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser						
60	65		70			
AAG ATT CCT AAG GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG						2990
Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser						
75	80		85			
ATG CTG TAC CTT GAC GAG AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG						3038
Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln						
90	95		100		105	
GAC ATG GTT GTG GAG GGT TGT GGG TGT CGC TAGTACAGCA AAATTAAATA						3088
Asp Met Val Val Glu Gly Cys Gly Cys Arg						
110	115					
CATAAAATATA	TATATATATA	TATATTTAG	AAAAAAGAAA	AAAATCTAGA	GTCGACCTGC	3148
AGTAATCGTA	CAGGGTAGTA	CAAATAAAAA	AGGCACGTCA	GATGACGTGC	CTTTTTCTT	3208
GTGAGCAGTA	AGCTTGGCAC	TGGCCGTGTC	TTTACAACGT	CGTGACTGGG	AAAACCTGG	3268
CGTTACCCAA	CTTAATCGCC	TTGCAGCACA	TCCCCCTTTC	GCCAGCTGGC	GTAATAGCGA	3328
AGAGGCCCGC	ACCGATCGCC	CTTCCCAACA	GTTGCGCAGC	CTGAATGGCG	AATGGCGCCT	3388

GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTCA CACCGCATAT ATGGTGCACT	3448
CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGC CCCGACACCC GCCAACACCC	3508
GCTGACGCCGC CCTGACGGGC TTGTCGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC	3568
GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGA	3623

## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 115 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys	
1 5 10 15	
Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp	
20 25 30	
Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu Cys	
35 40 45	
Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val	
50 55 60	
Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys	
65 70 75 80	
Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn	
85 90 95	
Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu Gly Cys	
100 105 110	
Gly Cys Arg	
115	

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 14 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CATGGGCAGC TGAG

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 41 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGGGTTGTG GGTGTCGCTA GTGAGTCGAC TACAGCAAAT T

41

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATGTGGGT GCCGCTGACT CTAGAGTCGA CGGAATTG

38

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AATTCAACCAT GATTCCCTGGT AACCGAATGC T

31

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTGGTACTAA GGACCATTGG CTTAC

25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGACCTGCAG CCATGCATCT GACTGTA

27

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGCCTGCAGT TTAATATTAG TGGCAGC

27

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGACCTGCAG CCACC

15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCGACCCACC ATGCCGGGGC TGGGGCGGAG GGCGCAGTGG CTGTGCTGGT GGTGGGGGCT 60  
GTGCTGCAGC TGCTGCGGGC C 81

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 73 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGCAGCAGCT GCACAGCAGC CCCCACCACC AGCACAGCCA CTGCGCCCTC CGCCCCAGCC 60  
CCGGCATGGT GGG 73

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 11 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCGACTGGTT T 11

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 9 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGAAACCA G

122

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCGACAGGGCT CGCCTGCA

18

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTCCGAGCGG

10

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CAGGTGACC CACCATGCAC GTGCGCTCA

29

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTGTCGACC TCGGAGGAGC TAGTGGC

## WHAT IS CLAIMED IS:

1. A method for producing a heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a sequence encoding a first selected BMP or fragment thereof and a sequence encoding a second selected BMP or fragment thereof, said sequences each being under the control of a suitable regulatory sequence capable of directing co-expression of said proteins, and isolating said heterodimeric protein from the culture medium.

2. The method according to claim 1 wherein said first BMP or fragment thereof is present on a first vector transfected into said host cell and said second BMP or fragment thereof is present on a second vector transfected into said host cell.

3. The method according to claim 1 wherein both said BMPs or fragments thereof are incorporated into a chromosome of said host cell.

4. The method according to claim 1 wherein both BMPs or fragments thereof are present on a single vector.

5. The method according to claim 2 wherein

more than a single copy of the gene encoding each said BMP or fragment thereof is present on each vector.

6. The method according to claim 1 wherein  
said host cell is a hybrid cell prepared by culturing two  
5 fused selected, stable host cells, each host cell  
transfected with a sequence encoding a selected first or  
second BMP or fragment thereof, said sequences under the  
control of a suitable regulatory sequence capable of  
directing expression of each protein or fragment.

10 7. The method according to claim 1 wherein  
said host cell is a mammalian cell.

8. The method according to claim 1 wherein  
said host cell is an insect cell.

15 9. The method according to claim 1 wherein  
said host cell is a yeast cell.

10. A method for producing a heterodimeric  
protein having bone stimulating activity in a bacterial  
cell comprising culturing a selected host cell containing  
a sequence encoding a first selected BMP or fragment  
thereof under the control of a suitable regulatory  
20 sequence capable of directing expression of the protein  
or protein fragment under conditions suitable for the

formation of a soluble, monomeric protein; culturing a selected host cell containing a sequence encoding a second selected BMP or fragment thereof under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under said conditions to form a second soluble, monomeric protein; and mixing said soluble monomeric proteins under conditions permitting the formation of dimeric proteins associated by at least one covalent disulfide bond; isolating from the mixture a heterodimeric protein.

11. The method according to claim 10 wherein said host cell is *E. coli*.

12. The method according to claim 10 wherein said conditions comprise treating said protein with a solubilizing agent.

13. A recombinant heterodimeric protein having bone stimulating activity comprising a first protein or fragment of BMP-2 in association with a second protein or fragment thereof selected from the group consisting of BMP-5, BMP-6, BMP-7 and BMP-8.

14. The protein according to claim 13 wherein said second protein is BMP-5.

15. The protein according to claim 13 wherein  
said second protein is BMP-6.

16. The protein according to claim 13 wherein  
said second protein is BMP-7.

5 17. The protein according to claim 13 wherein  
said second protein is BMP-8.

18. A recombinant heterodimeric protein having  
bone stimulating activity comprising a protein or  
fragment of BMP-4 in association with a second protein or  
10 fragment thereof selected from the group consisting of  
BMP-5, BMP-6, BMP-7 and BMP-8.

19. The protein according to claim 18 wherein  
said second protein is BMP-5.

20. The protein according to claim 18 wherein  
15 said second protein is BMP-6.

21. The protein according to claim 18 wherein  
said second protein is BMP-7.

22. The protein according to claim 18 wherein  
said second protein is BMP-8.

23. A recombinant heterodimeric protein having  
bone stimulating activity comprising a protein or  
fragment of a first BMP in association with a second  
protein or fragment of a second BMP produced by co-  
5 expressing said proteins in a selected host cell.

24. The protein according to claim 23 wherein  
said first BMP is BMP-2 and said second BMP is BMP-7.

25. A cell line comprising a nucleotide  
sequence encoding a first BMP or fragment thereof under  
10 control of a suitable expression regulatory system and a  
nucleotide sequence encoding a second BMP or fragment  
thereof under control of a suitable expression regulatory  
system, said regulatory systems capable of directing the  
co-expression of said BMPs or fragments thereof and the  
15 formation of heterodimeric protein.

26. The cell line according to claim 25  
wherein said nucleotide sequences encoding said first and  
second BMP proteins are present in a single DNA molecule.

27. The cell line according to claim 25  
20 wherein said nucleotide sequence encoding said first BMP  
is present on a first DNA molecule and said nucleotide  
sequence encoding said second BMP is present on a second  
DNA molecule.

28. The cell line according to claim 26  
wherein said single DNA molecule comprises a first  
transcription unit containing a gene encoding a first BMP  
or fragment thereof and a second transcription unit  
5 containing a gene encoding a second BMP or fragment  
thereof.

29. The cell line according to claim 26  
wherein said single DNA molecule comprises a single  
transcription unit containing multiple copies of said  
10 gene encoding said first BMP or fragments thereof and  
multiple copies of said gene encoding said second BMP or  
fragments thereof.

30. A DNA molecule comprising a sequence  
encoding a first selected BMP or fragment thereof and a  
15 sequence encoding a second selected BMP or fragment  
thereof, said sequences under the control of at least one  
suitable regulatory sequence capable of directing co-  
expression of each BMP or fragment thereof.

31. The molecule according to claim 30  
20 comprising a first transcription unit containing a gene  
encoding a first BMP or fragment thereof and a second  
transcription unit containing a gene encoding a second  
BMP or fragment thereof.

32. The molecule according to claim 30 comprising a single transcription unit containing multiple copies of said gene encoding said first BMP or fragments thereof and multiple copies of said gene encoding said second BMP or fragments thereof.

5

33. The protein according to claim 23 wherein said first BMP is BMP-2 and said second BMP is BMP-6.

34. A recombinant BMP-2 homodimer having bone stimulating activity said homodimer produced in E. coli.

10

35. A method for producing a homodimeric BMP-2 protein having bone stimulating activity said method comprising culturing E. coli host cells and isolating and purifying said protein from the resulting culture medium.

15

36. A recombinant heterodimeric protein having bone stimulating activity comprising a first protein or fragment of BMP-2 in association with a second protein or fragment of BMP-2.

## FIGURE 1A

10 20 30 40 50 60 70  
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACCTTG CAGGGAGAAAT AACTTGCAGCA  
 80 90 100 110 120 130 140  
 CCCCCACTTTG CGCCGGTGCC TTTGCCAG CGGAGCCTGC TTGCCATCT CCGAGCCCCA CGGCCCTCC  
 150 160 170 180 190 200 210  
 ACTCCCTCGGC CTGCCCCGAC ACTGAGACGC TGTTCCCAGC GTGAAAAGAG AGACTGCGCG GCCGGCACCC  
 220 230 240 250 260 270 280  
 GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTGGT CCTTGCGCCA GGTCCATTGA CCAGAGTTT  
 290 300 310 320 330 340 350  
 TCCATGTGGA CGCTCTTCA ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT  
  
 (1) 370 385 400  
 CGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Pro Gln Val  
 415 430 445  
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 (24)  
 460 475 490 505  
 GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 520 535 550 565  
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 580 595 610  
 AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCA GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 625 640 655 670  
 CAG CCG GGC TCA CCC GCC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

## **SUBSTITUTE SHEET**

## FIGURE 1B

685 700 715  
 AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Leu Pro Glu Thr  
  
 730 745 760 775  
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
  
 850 865 880 895  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
  
 895 910 925 940  
 CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp Thr Arg Leu  
  
 955 970 985 995  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
  
 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
  
 1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 (249)  
  
 1120 1135 1150 1165  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 (266)  
  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 (283)  
  
 1225 1240 1255 1270  
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 (296)  
  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala

## SUBSTITUTE SHEET

**FIGURE 1C**

1330	1345	1360	1375			
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT						
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr						
1390	1405	1420				
AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT AAC TCT AAG ATT CCT AAG						
Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys						
1435	1450	1465	1480			
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG						
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu						
1495	1510	1525				
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG						
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly						
1540(396)	1553	1563	1573	1583	1593	1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTAG AAAAAAGAAA						
Cys Arg						

AAAA

**FIGURE 2A**

10 20 30 40 50 60 70  
 CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CGGAAAGCTA GGTGAGTGTG  
 80 90 100 110 120 130 140  
 GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC  
 150 160 170 180 190 200 210  
 GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG  
 220 230 240 250 260 270 280  
 CAACC GTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC  
 290 300 310 320 330 340 350  
 GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTGT TCAAGATTGG  
 360 370 380 390 400 (1)  
 CTGTCAAGAAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT  
 MET Ile Pro  
 417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 477 492 507  
 AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Val Ala Glu Ile Gln  
 522 537 552 567  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 642 657 672  
 AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

## FIGURE 2B

687                    702                    717                    732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG CCG GCC  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
  
 747                    762                    777  
 AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
  
 792                    807                    822                    837  
 CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
  
 852                    867                    882                    897  
 CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
  
 912                    927                    942  
 GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
  
 957                    972                    987                    1002  
 ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
  
 1017                  1032                  1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
  
 1062                  1077                  1092                  1107  
 GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
  
 1122                  1137                  1152                  1167  
 GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser  
  
 1182                  1197                  1212  
 CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
  
 1227                  1242                  1257                  1272  
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys  
  
 1287                  1302                  1317  
 CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 (293)

## FIGURE 2C

1332                  1347                  1362                  1377  
CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

1392                  1407                  1422                  1437  
GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

1452                  1467                  1482  
GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497                  1512                  1527                  1542  
GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC  
Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

1557                  1572                  1587  
TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

1602                  1617                  (408)                  1636                  1646                  1656  
ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG  
MET Val Val Glu Gly Cys Gly Cys Arg

1666                  1676                  1686                  1696                  1706                  1716                  1726  
ATATACACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC

1736                  1746                  1756                  1766                  1776                  1786                  1796  
ACAGACTGCT TCCTTATAGC TGGACTTTA TTTAAAAAAA AAAAAAAA AATGGAAAAA ATCCCTAAAC

1806                  1816                  1826                  1836                  1846                  1856                  1866  
ATTCACTTGT ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA

1876                  1886                  1896                  1906                  1916                  1926                  1936  
ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAAACT

1946  
CTAGAGTCGA CGGAATTC

## **FIGURE 3A**

10	20	30	40	50
GTGACCGAGC	GGCGCGGACG	GCCGCCTGCC	CCCTCTGCCA	CCTGGGGCGG
60	70	80	90	99
TGCGGGGCCG	GAGCCCAGG	CCCGGGTAGC	GCGTAGAGCC	GGCGCG ATG MET (1)
108	117	126	135	144
CAC GTG CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG				
His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala				
153	162	171	180	189
CTC TGG GCA CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC				
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe				
198	207	216	225	234
AGC CTG GAC AAC GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC				
Ser Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu				
243	252	261	270	279
CGC AGC CAG GAG CGG CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT				
Arg Ser Gln Glu Arg Arg Glu MET Gln Arg Glu Ile Leu Ser Ile				
288	297	306	315	324
TTG GGC TTG CCC CAC CGC CCG CGC CCG CAC CTC CAG GGC AAG CAC				
Leu Gly Leu Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His				
333	342	351	360	369
AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG TAC AAC GCC ATG GCG				
Asn Ser Ala Pro MET Phe MET Leu Asp Leu Tyr Asn Ala MET Ala				
378	387	396	405	414
GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC TTC TCC TAC CCC				
Val Glu Glu Gly Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro				
423	432	441	450	459
TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG GCC AGC CTG				
Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu Ala Ser Leu				
468	477	486	495	504
CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG AGC TTC				
Gln Asp Ser His Phe Leu Thr Asp Ala Asp MET Val MET Ser Phe				
513	522	531	540	549
GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC TAC				
Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg Tyr				

## FIGURE 3B

558	567	576	585	594										
CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCA	GAA	GGG
His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	Gly
603	612	621	630	639										
GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	TAC	ATC
Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile
648	657	666	675	684										
CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	CAG
Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	Gln
693	702	711	720	729										
GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC
Val	Leu	Gln	Leu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu
738	747	756	765	774										
GAC	AGC	CGT	ACC	CFC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT
Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe
783	792	801	810	819										
GAC	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC
Asp	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His
828	837	846	855	864										
AAC	CTG	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC
Asn	Leu	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser
873	882	891	900	909										
ATC	AAC	CCC	AAG	TTG	GGC	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG
Ile	Asn	Pro	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln
918	927	936	945	954										
AAC	AAG	CAG	CCC	TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC
Asn	Lys	Pro	Phe	MET	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	
963	972	981	990	999										
CAC	TTC	CGC	AGC	ATC	CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG
His	Phe	Arg	Ser	Ile	Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
(293)														
1008	1017	1026	1035	1044										
AAC	CGC	TCC	AAG	ACG	CCC	AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln	Glu	Ala	Leu	Arg	MET	Ala
1053	1062	1071	1080	1089										
AAC	GTG	GCA	GAG	AAC	AGC	AGC	AGC	GAC	CAG	AGG	CAG	GCC	TGT	AAG
Asn	Val	Ala	Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys

## FIGURE 3C

1098	1107	1116	1125	1134
AAG CAC GAG CTG TAT GTC AGC TTC CGA GAC CTG GGC TGG CAG GAC				
Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp				
1143	1152	1161	1170	1179
TGG ATC ATC GCG CCT GAA GGC TAC GCC GCC TAC TAC TGT GAG GGG				
Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly				
1188	1197	1206	1215	1224
GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC ACC AAC CAC				
Glu Cys Ala Phe Pro Leu Asn Ser Tyr MET Asn Ala Thr Asn His				
1233	1242	1251	1260	1269
GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC CCG GAA ACG GTG				
Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Ile Ser Val				
1278	1287	1296	1305	1314
CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC ATC TCC GTC				
Pro Lys Pro Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val				
1323	1332	1341	1350	1359
CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA TAC AGA				
Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg				
1368	1377	1386	1399	
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC				
Asn MET Val Val Arg Ala Cys Gly Cys His				
(431)				
1409	1419	1429	1439	1448
GAGAATTTCAG ACCCTTGCG GCCAAGTTT TCTGGATCCT CCATTGCTC				

10/32

## FIGURE 4A

10            20            30            40            50  
 CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC

60            70            80            90            100  
 GAGAGGTGGC GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG

110            120            130            140            150  
 GCCTCGCTCC GCCTCGCTCCAC GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC

159            168            177            186            195  
 CGGGCGGGGG ATG CCG GGG CTG GGG CGG AGG GCG CAG TGG CTG TGC  
 MET Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys  
 (1)

204            213            222            231            240  
 TGG TGG TGG GGG CTG CTG TGC AGC TGC TGC GGG CCC CCG CCG CTG  
 Trp Trp Trp Gly Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu

249            258            267            276            285  
 CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC GCC GGG GGG CAG  
 Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala Ala Gly Gly Gln

294            303            312            321            330  
 CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG GAG CAG CCG CCG  
 Leu Leu Gly Asp Gly Ser Pro Gly Arg Thr Glu Gln Pro Pro

339            348            357            366            375  
 CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG CGG CTC AAG  
 Pro Ser Pro Gln Ser Ser Gly Phe Leu Tyr Arg Arg Leu Lys

384            393            402            411            420  
 ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG GTG CTG  
 Thr Gln Glu Lys Arg Glu MET Gln Lys Glu Ile Leu Ser Val Leu

429            438            447            456            465  
 GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG CCG  
 Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln Pro

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**FIGURE 4B**

474	483	492	501	510
CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG	Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu Gln Gln Gln Gln			
519                528                537                546                555				
CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG	Gln Leu Pro Arg Gly Glu Pro Pro Gly Arg Leu Lys Ser Ala			
564                573                582                591                600				
CCC CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC	Pro Leu Phe MET Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn			
609                618                627                636                645				
GAC GAG GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC	Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro			
654                663                672                681                690				
CAC GAA GCA GCC AGC TCG TCC CAG CGT CGG CAG CCG CCC CCG GGC	His Glu Ala Ala Ser Ser Gln Arg Arg Gln Pro Pro Gly Ser			
699                708                717                726                735				
GCC GCG CAC CCG CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT	Pro Pro Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala			
744                753                762                771                780				
GGC AGC GGC GGC GCG TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC	Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala			
789                798                807                816                825				
TTC CTC AAC GAC GCG GAC ATG GTC ATG AGC TTT GTG AAC CTG GTG	Phe Leu Asn Asp Ala Asp MET Val MET Ser Phe Val Asn Leu Val			
834                843                852                861                870				
GAG TAC GAC AAG GAG TTC TCC CCT CGT CAG CGA CAC CAC AAA GAG	Glu Tyr Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu			
879                888                897                906                915				
TTC AAG TTC AAC TTA TCC CAG ATT CCT GAG GGT GAG GTG GTG ACG	Phe Lys Phe Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr			
924                933                942                951                960				
GCT GCA GAA TTC CGC ATC TAC AAG GAC TGT GTT ATG GGG AGT TTT	Phe Arg Ile Tyr Lys Asp Cys Val MET Ala Ala Glu Gly Ser Phe			

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## FIGURE 4C

969            978            987            996            1005  
 AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT CAA GTC TTA CAG GAG  
 Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu

1014            1023            1032            1041            1050  
 CAT CAG CAC AGA GAC TCT GAC CTG TTT TTG TTG GAC ACC CGT GTA  
 His Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg Val

1059            1068            1077            1086            1095  
 GTA TGG GCC TCA GAA GAA GGC TGG CTG GAA TTT GAC ATC ACG GCC  
 Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala

1104            1113            1122            1131            1140  
 ACT AGC AAT CTG TGG GTT GTG ACT CCA CAG CAT AAC ATG GGG CTT  
 Thr Ser Asn Leu Trp Val Val Thr Pro Gln His Asn MET Gly Leu

1149            1158            1167            1176            1185  
 CAG CTG AGC GTG GTG ACA AGG GAT GGA GTC CAC GTC CAC CCC CGA  
 Gln Leu Ser Val Val Thr Arg Asp Gly Val His Val His Pro Arg

1194            1203            1212            1221            1230  
 GCC GCA GGC CTG GTG GGC AGA GAC GGC CCT TAC GAT AAG CAG CCC  
 Ala Ala Gly Leu Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro

1239            1248            1257            1266            1275  
 TTC ATG GTG GCT TTC TTC AAA GTG AGT GAG GTC CAC GTG CGC ACC  
 Phe MET Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg Thr

1284            1293            1302            1311            1320  
 ACC AGG TCA GCC TCC AGC CGG CGC CGA CAA CAG AGT CGT AAT CGC  
 Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser Arg Asn Arg  
 (382)

1329            1338            1347            1356            1365  
TCT ACC CAG TCC CAG GAC GTG GCG CGG GTC TCC AGT GCT TCA GAT  
Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala Ser Asp  
 (388)

1374            1383            1392            1401            1410  
 TAC AAC AGC AGT GAA TTG AAA ACA GCC TGC AGG AAG CAT GAG CTG  
 Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu Leu  
 (412)

1419            1428            1437            1446            1455  
TAT GTG AGT TTC CAA GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA  
Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala

## FIGURE 4D

1464            1473            1482            1491            1500  
 CCC AAG GGC TAT GCT GCC AAT TAC TGT GAT GGA GAA TGC TCC TTC  
 Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe

1509            1518            1527            1536            1545  
 CCA CTC AAC GCA CAC ATG AAT GCA ACC AAC CAC GCG ATT GTG CAG  
 Pro Leu Asn Ala His MET Asn Ala Thr Asn His Ala Ile Val Gln

1554            1563            1572            1581            1590  
 ACC TTG GTT CAC CTT ATG AAC CCC GAG TAT GTC CCC AAA CCG TGC  
 Thr Leu Val His Leu MET Asn Pro Glu Tyr Val Pro Lys Pro Cys

1599            1608            1617            1626            1635  
 TGT GCG CCA ACT AAG CTA AAT GCC ATC TCG GTT CTT TAC TTT GAT  
 Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp

1644            1653            1662            1671            1680  
 GAC AAC TCC AAT GTC ATT CTG AAA AAA TAC AGG AAT ATG GTT GTA  
 Asp Asn Ser Asn Val Ile Leu Lys Tyr Arg Asn MET Val Val

1689            1698            1708            1718            1728  
 AGA GCT TGT GGA TGC CAC TAACTCGAAA CCAGATGCTG GGGACACACA  
 Arg Ala Cys Gly Cys His  
 (513)

1738            1748            1758            1768            1778  
 TTCTGCCTTG GATTCCCTAGA TTACATCTGC CTTAAAAAAA CACGGAAGCA

1788            1798            1808            1818            1828  
 CAGTTGGAGG TGGGACGATG AGACTTTGAA ACTATCTCAT GCCAGTGCCT

1838            1848            1858            1868            1878  
 TATTACCCAG GAAGATTTA AAGGACCTCA TTAATAATTT GCTCACTTGG

1888            1898            1908            1918            1928  
 TAAATGACGT GAGTAGTTGT TGGTCTGTAG CAAGCTGAGT TTGGATGTCT

1938            1948            1958            1968            1978  
 GTAGCATAAG GTCTGGTAAC TGCAGAAACA TAACCCTGAA GCTCTTCCTA

1988            1998            2008            2018            2028  
 CCCTCCTCCC CCAAAACCC ACCAAAATTA GTTTAGCTG TAGATCAAGC

2038            2048            2058            2068            2078  
 TATTGGGGT GTTTGTTAGT AAATAGGGAA AATAATCTCA AAGGAGTTAA

2088            2098            2108            2118            2128  
 ATGTATTCTT GGCTAAAGGA TCAGCTGGTT CAGTACTGTC TATCAAAGGT

## FIGURE 4E

2138            2148            2158            2168            2178  
 AGATTTACA GAGAACAGAA ATCGGGGAAG TGGGGGAAAC GCCTCTGTTC  
  
 2188            2198            2208            2218            2228  
 AGTTCAATTCC CAGAAGTCCA CAGGACGCAC AGCCCAGGCC ACAGCCAGGG  
  
 2238            2248            2258            2268            2278  
 CTCCACGGGG CGCCCTTGTC TCAGTCATTG CTGTTGTATG TTCGTGCTGG  
  
 2288            2298            2308            2318            2328  
 AGTTTTGTTG GTGTGAAAAT ACACTTATTG CAGCCAAAAC ATACCATTTC  
  
 2338            2348            2358            2368            2378  
 TACACCTCAA TCCTCCATTG GCTGTACTCT TTGCTAGTAC CAAAAGTAGA  
  
 2388            2398            2408            2418            2428  
 CTGATTACAC TGAGGTGAGG CTACAAGGGG TGTGTAACCG TGTAACACGT  
  
 2438            2448            2458            2468            2478  
 GAAGGCAGTG CTCACCTCTT CTTTACCAAGA ACGGTTCTTT GACCAGCACA  
  
 2488            2498            2508            2518            2528  
 TTAACCTCTG GACTGCCGGC TCTAGTACCT TTTCAGTAAA GTGGTTCTCT  
  
 2538            2548            2558            2568            2578  
 GCCTTTTAC TATAACAGCAT ACCACGCCAC AGGGTTAGAA CCAACGAAGA  
  
 2588            2598            2608            2618            2628  
 AAATAAAATG AGGGTGCCCA GCTTATAAGA ATGGTGTAG GGGGATGAGC  
  
 2638            2648            2658            2668            2678  
 ATGCTGTTA TGAACGGAAA TCATGATTTC CCTGTAGAAA GTGAGGCTCA  
  
 2688            2698            2708            2718            2728  
 GATTAATTT TAGAATATTG TCTAAATGTC TTTTCACAA TCATGTGACT  
  
 2738            2748            2758            2768            2778  
 GGGAAAGGCAA TTTCATACTA AACTGATTAA ATAATACATT TATAATCTAC  
  
 2788            2798            2808            2818            2828  
 AACTGTTGC ACTTACAGCT TTTTTGTAA ATATAAACTA TAATTTATTG  
  
 2838            2848            2858            2868            2878  
 TCTATTATTAT ATCTGTTTG CTGTGGCGTT GGGGGGGGGG CCGGGGCTTTT  
  
 2888            2898            2908            2918  
 GGGGGGGGGG GTTTGTTGG GGGGTGTCGT GGTGTGGCG GGCAGG

## FIGURE 5A

10            20            30            40            50  
 CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA  
 60            70            80            90            100  
 GGGATTGAAT GGACTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC  
 110           120           130           140           150  
 ATTTACTTGATAGTACAAC CTAGAGTATT ATTTCACACT AAGACGACAC  
 160           170           180           190           200  
 AAAAGATGTT AAAGTTATCA CCAAGCTGCC GGACAGATAT ATATTCCAAC  
 210           220           230           240           250  
 ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC AGGATTTGTT  
 260           270           280           290           300  
 TTGGAAAGAG CTCAAGGGTT GAGAAGAACT CAAAAGCAAG TGAAGATTAC  
 310           320           330           340           350  
 TTTGGAAACT ACAGTTTATC AGAACATCAA CTTTGCTAA TTCAAATACC  
 360           370           380           390           400  
 AAAGGCCTGA TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC  
 410           420           430           440           450  
 AAATAATATT AGCCGTCTTC TGCTACATCA ATGCAGCAAA AACTCTAAC  
 460           470           480           490           500  
 AACTGTGGAT AATTGGAAAT CTGAGTTCA GCTTTCTTAG AAATAACTAC  
 510           520           530           540           550  
 TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAA TCGGTGAGGA  
 560           570           580           590           600  
 TGTTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT  
 610           620           630           640           650  
 TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTT  
 660           670           680           690           700  
 AAGAGGACAA GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAA

## FIGURE 5B

701	710	719	728	737										
ATG	CAT	CTG	ACT	GTA	TTT	TTA	CTT	AAG	GGT	ATT	GTG	GGT	TTC	CTC
MET	His	Leu	Thr	Val	Phe	Leu	Leu	Lys	Gly	Ile	Val	Gly	Phe	Leu
(1)														
746	755	764	773	782										
TGG	AGC	TGC	TGG	GTT	CTA	GTG	GGT	TAT	GCA	AAA	GGA	GGT	TTG	GGA
Trp	Ser	Cys	Trp	Val	Leu	Val	Gly	Tyr	Ala	Lys	Gly	Gly	Leu	Gly
791	800	809	818	827										
GAC	AAT	CAT	GTT	CAC	TCC	AGT	TTT	ATT	TAT	AGA	AGA	CTA	CGG	AAC
Asp	Asn	His	Val	His	Ser	Ser	Phe	Ile	Tyr	Arg	Arg	Arg	Leu	Asn
836	845	854	863	872										
CAC	GAA	AGA	CGG	GAA	ATA	CAA	AGG	GAA	ATT	CTC	TCT	ATC	TTG	GGT
His	Glu	Arg	Arg	Glu	Ile	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly
881	890	899	908	917										
TTG	CCT	CAC	AGA	CCC	AGA	CCA	TTT	TCA	CCT	GGA	AAA	ATG	ACC	AAT
Leu	Pro	His	Arg	Pro	Arg	Pro	Phe	Ser	Pro	Gly	Lys	Gln	Ala	Ser
926	935	944	953	962										
CAA	GCG	TCC	TCT	GCA	CCT	CTC	TTT	ATG	CTG	GAT	CTC	TAC	AAT	GCC
Ser	Ala	Pro	Leu	Phe	MET	Leu	Asp	Leu	Tyr	Asn	Ala	MET	Thr	Asn
971	980	989	998	1007										
GAA	GAA	AAT	CCT	GAA	GAG	TCG	GAG	TAC	TCA	GTA	AGG	GCA	TCC	TTG
Glu	Glu	Asn	Pro	Glu	Glu	Ser	Glu	Tyr	Ser	Val	Arg	Ala	Ser	Leu
1016	1025	1034	1043	1052										
GCA	GAA	GAG	ACC	AGA	GGG	GCA	AGA	AAG	GGA	TAC	CCA	GCC	TCT	CCC
Ala	Glu	Glu	Thr	Arg	Gly	Ala	Arg	Lys	Gly	Tyr	Pro	Ala	Ser	Pro
1061	1070	1079	1088	1097										
AAT	GGG	TAT	CCT	CGT	CGC	ATA	CAG	TTA	TCT	CGG	ACG	ACT	CCT	CTG
Asn	Gly	Tyr	Pro	Arg	Arg	Ile	Gln	Leu	Ser	Arg	Thr	Thr	Pro	Leu
1106	1115	1124	1133	1142										
ACC	ACC	CAG	AGT	CCT	CCT	CTA	GCC	AGC	CTC	CAT	GAT	ACC	AAC	TTT
Thr	Thr	Gln	Ser	Pro	Pro	Leu	Ala	Ser	Leu	His	Asp	Thr	Asn	Phe
1151	1160	1169	1178	1187										
CTG	AAT	GAT	GCT	GAC	ATG	GTC	ATG	AGC	TTT	GTC	AAC	TTA	GTT	GAA
Leu	Asn	Asp	Ala	Asp	MET	MET	Ser	Phe	Val	Asn	Leu	Val	Glu	
1196	1205	1214	1223	1232										
AGA	GAC	AAG	GAT	TTT	TCT	CAC	CAG	CGA	AGG	CAT	TAC	AAA	GAA	TTT
Arg	Asp	Lys	Asp	Phe	Ser	His	Gln	Arg	Arg	His	Tyr	Lys	Glu	Phe

## FIGURE 5C

1241	1250	1259	1268	1277
CGA TTT GAT CTT ACC CAA ATT CCT CAT GGA GAG GCA GTG ACA GCA				
Arg Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala Val Thr Ala				
1286	1295	1304	1313	1322
GCT GAA TTC CGG ATA TAC AAG GAC CGG AGC AAC AAC CGA TTT GAA				
Ala Glu Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg Phe Glu				
1331	1340	1349	1358	1367
AAT GAA ACA ATT AAG ATT AGC ATA TAT CAA ATC ATC AAG GAA TAC				
Asn Glu Thr Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu Tyr				
1376	1385	1394	1403	1412
ACA AAT AGG GAT GCA GAT CTG TTC TTG TTA GAC ACA AGA AAG GCC				
Thr Asn Arg Asp Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala				
1421	1430	1439	1448	1457
CAA GCT TTA GAT GTG GGT TGG CTT GTC TTT GAT ATC ACT GTG ACC				
Gln Ala Leu Asp Val Gly Trp Leu Val Phe Asp Ile Thr Val Thr				
1466	1475	1484	1493	1502
AGC AAT CAT TGG GTG ATT AAT CCC CAG AAT AAT TTG GGC TTA CAG				
Ser Asn His Trp Val Ile Asn Pro Gln Asn Asn Leu Gly Leu Gln				
1511	1520	1529	1538	1547
CTC TGT GCA GAA ACA GGG GAT GGA CGC AGT ATC AAC GTA AAA TCT				
Leu Cys Ala Glu Thr Gly Asp Gly Arg Ser Ile Asn Val Lys Ser				
1556	1565	1574	1583	1592
GCT GGT CTT GTG GGA AGA CAG GGA CCT CAG TCA AAA CAA CCA TTC				
Ala Gly Leu Val Gly Arg Gln Gly Pro Gln Ser Lys Gln Pro Phe				
1601	1610	1619	1628	1637
ATG GTG GCC TTC TTC AAG GCG AGT GAG GTA CTT CTT CGA TCC GTG				
MET Val Ala Phe Phe Lys Ala Ser Glu Val Leu Leu Arg Ser Val				
1646	1655	1664	1673	1682
AGA GCA GCC AAC AAA CGA AAA AAT CAA AAC CGC AAT AAA TCC AGC				
Arg Ala Ala Asn Lys Arg Lys Asn Gln Asn Arg Asn Lys Ser Ser				
(329)				
1691	1700	1709	1718	1727
TCT CAT CAG GAC TCC TCC AGA ATG TCC AGT GTT GGA GAT TAT AAC				
<u>Ser His Gln Asp Ser Ser Arg MET Ser Ser Val Gly Asp Tyr Asn</u>				
(337)				

18/32

## FIGURE 5D

1736	1745	1754	1763	1772	
ACA AGT GAG CAA AAA CAA GCC TGT AAG AAG CAC GAA CTC TAT GTG					
Thr Ser Glu Gln Lys Gln Ala Cys Lys Lys <u>His</u> Glu Leu Tyr Val					
(356)					
1781	1790	1799	1808	1817	
AGC TTC CGG GAT CTG GGA TGG CAG GAC TGG ATT ATA GCA CCA GAA					
<u>Ser</u> <u>Phe</u> Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu					
(362)					
1826	1835	1844	1853	1862	
GGA TAC GCT GCA TTT TAT TGT GAT GGA GAA TGT TCT TTT CCA CTT					
Gly Tyr Ala Ala Phe Tyr Cys Asp Gly Glu Cys Ser Phe Pro Leu					
1871	1880	1889	1898	1907	
AAC GCC CAT ATG AAT GCC ACC AAC CAC GCT ATA GTT CAG ACT CTG					
Asn Ala His MET Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu					
1916	1925	1934	1943	1952	
GTT CAT CTG ATG TTT CCT GAC CAC GTA CCA AAG CCT TGT TGT GCT					
Val His Leu MET Phe Pro Asp His Val Pro Lys Pro Cys Cys Ala					
1961	1970	1979	1988	1997	
CCA ACC AAA TTA AAT GCC ATC TCT GTT CTG TAC TTT GAT GAC AGC					
Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser					
2006	2015	2024	2033	2042	
TCC AAT GTC ATT TTG AAA AAA TAT AGA AAT ATG GTA GTA CGC TCA					
Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn MET Val Val Arg Ser					
2051	2060	2070	2080	2090	2100
TGT GGC TGC CAC TAATATTAAA TAATATTGAT AATAACAAAA AGATCTGTAT					
Cys Gly Cys His					
(454)					
2110	2120	2130	2140	2150	
TAAGGTTAT GGCTGCAATA AAAAGCATAAC TTTCAGACAA ACAGAAAAAA AAA					

Figure 6

(1)  
GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT  
Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala  
(10)

GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC  
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His  
(20) (30)

CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC  
Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser  
(40) (50)

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT GGA GAC  
Asn Arg Glu Ser Asp Leu Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp  
(60) (70)

GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG  
Glu Gly Typ Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cyc Trp Leu Leu Lys  
(80)

CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC  
Arg His Lys Asp Leu Gly Lue Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser  
(90) (100)

GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG  
Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln  
(110) (120)

CCT TTC GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG  
Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg  
(130) (140)

GCA GTG AGG CCA CTG AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG  
Ala Val Arg Pro Leu Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln  
(150) (160)

GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG  
Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln  
(170)

GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTT GGC TGG CTG GAC  
Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp  
(180) (190)

TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAG GGG GAG TGC TCC  
Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser  
(200) (210)

TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG  
Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu  
(220) (230)

## Figure 6 (Con't)

GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG  
Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys  
(240) (250)

CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC  
Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg  
(260)

AAG CAC CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCCCCGCCAGC  
Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
(270) (280)

CCTACTGCAGCCACCCCTCTCATCTGGATGGGGCCCTGCAGAGGCAGAAAAACCTTAAATGCTGTACAG  
CTCAAGCAGGAGTGTCAAGGGGCCCTCACTCTCGGTGCCTACTTCCTGTCAGGCTTCTGGGAATTG

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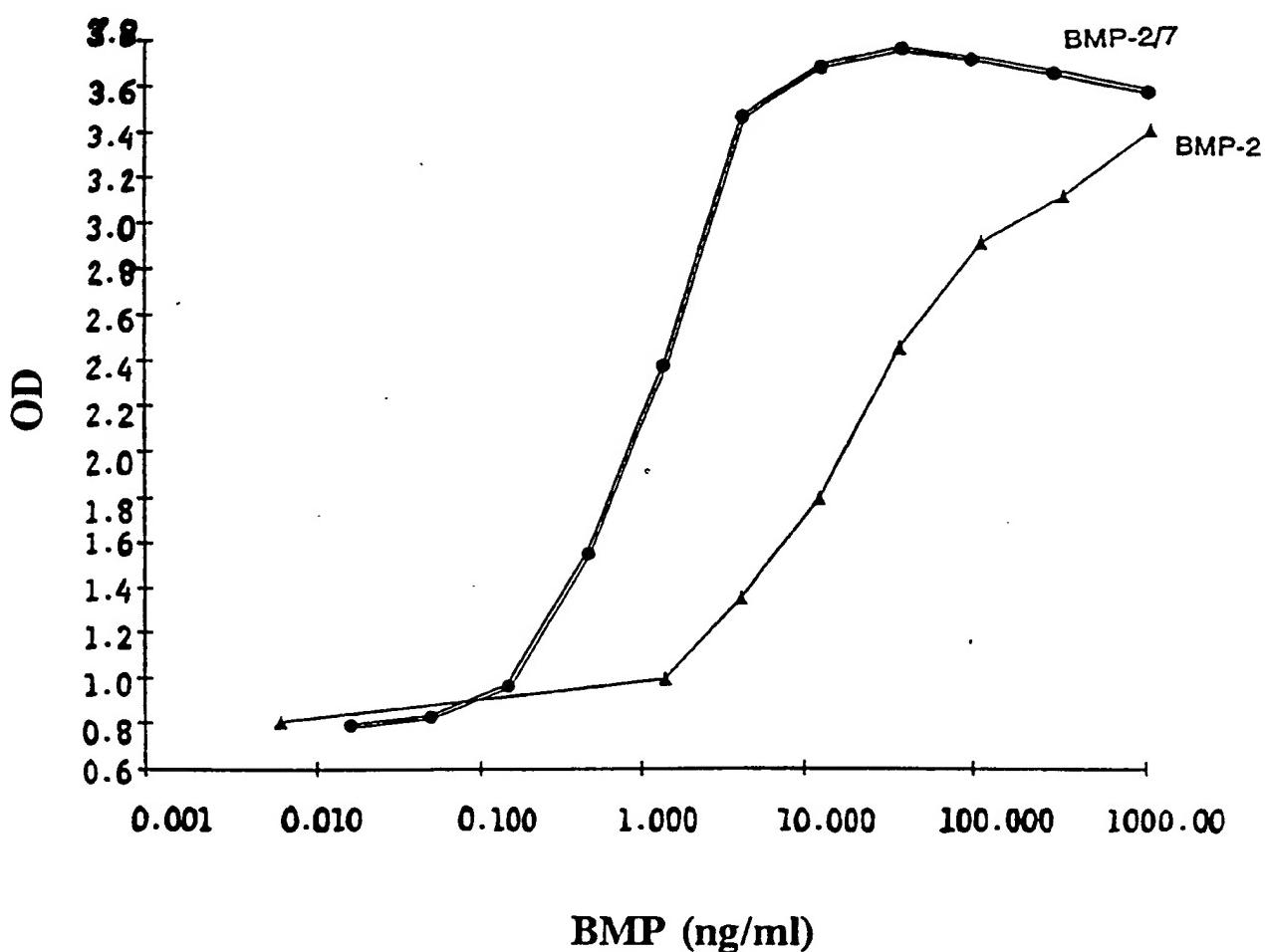
## FIGURE 7

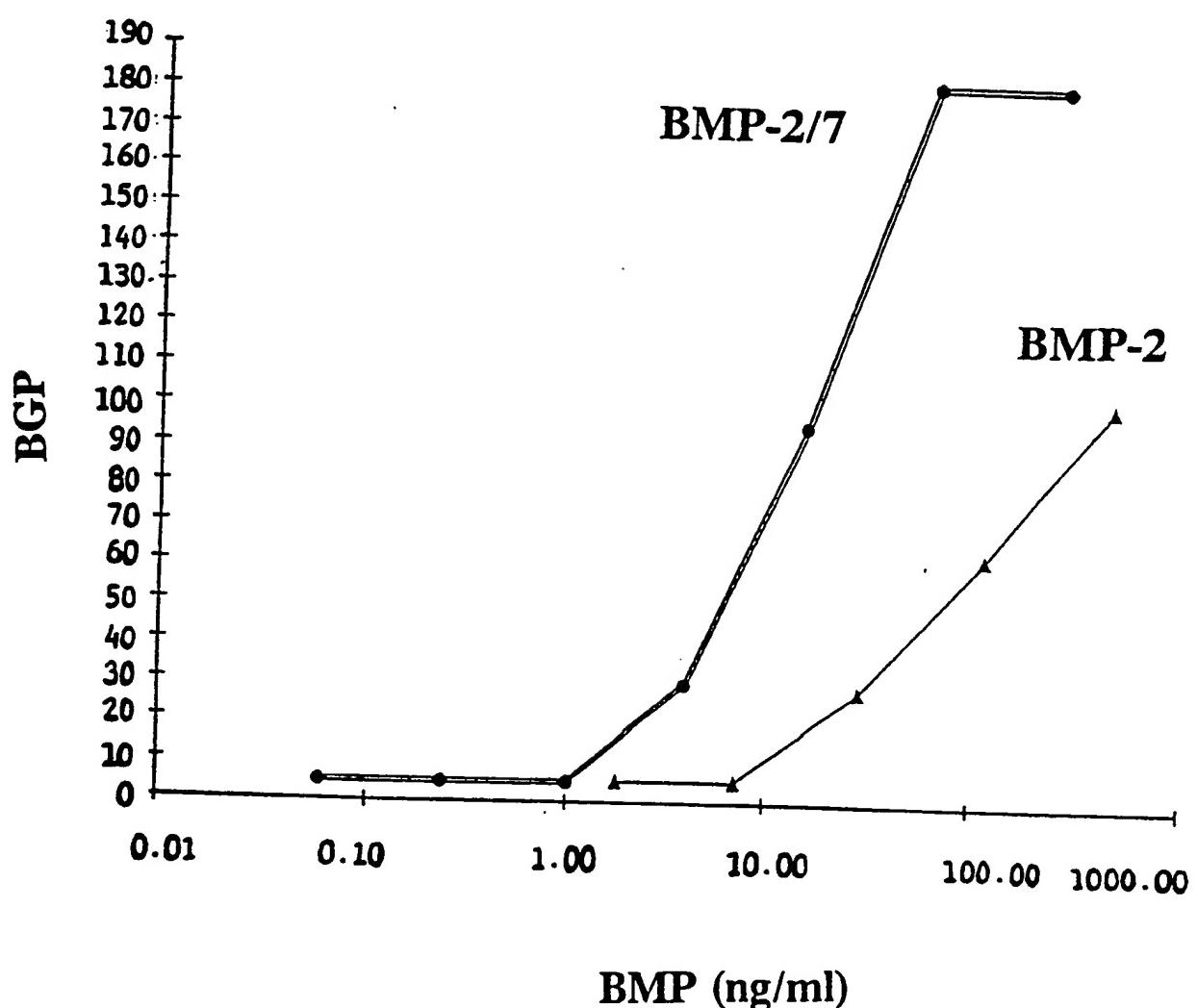
GACCAAAAGGG CCTCGTGATA CGCCTATTT TATAAGTTAA TGTCA TGATA ATAATGGTTT 60  
 CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCCGG AACCCCTATT TGTTTATTT 120  
 TCTAAATACA TTCAAAATATG TATCCGCTCA TGAGACAAATA ACCCTGATAAA ATGCTTCAT 180  
 AATATTGAAA AAGGAAGACT ATGAGTATTC AACATTTCGG TGTGGCCCTT ATTCCCTTTT 240  
 TTGCGGGATT TTGCCTTCCT GTTTTGCTC ACCCAGAAAC GCTGGTGAAA CTAAAAGATG 300  
 CTGAAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAAGA 360  
 TCCTTGAGAG TTTTCGGCCCG GAAGAACGTT TTCCAATGAT GAGCACTTTT AAGGTTCTGC 420  
 TATGTCGGCCG CGTATTATCC CGTATTGACG CGGGGCGAGA GCAACTCGGT CGCCCGCATAC 480  
 ACTATTCTCA CAATGACTTG GTTGAGTACT CACCA GTGAG AGAAAAGCAT CTTACGGATG 520  
 GCATGACAGT AAGAGAATTA TGCAGTGCTG CGATAACCAT GAGTGATAAAC ACTGGGGCCA 580  
 ACTTACTTCT GACAAAGGATC CGAGGACCGA AGGAGCTAAC CGCTTTTTG CACAACTGG 660  
 CGGATCATGT AACTGGCTT GATGTTGGG ACCCGGAGCT GATGAAAGCC ATACCAAACG 720  
 ACCAGGCTGA CACCAGGATG CCTGTAGCAA TGGCAACAAAC GTTGCGCAAA CTATTAACG 780  
 CGGAACTACT TACTCTAGCT TCCGGCAAC AATTAATAGA CTGGATGGAG CGGGATAAAG 840  
 TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAAATCTG 900  
 GAGCCCCGTGA CGCTGGGTCT CGCGGTATCA TTGAGGACT CGGGCCAGAT CGTAAAGCCCT 960  
 CCCGTATCGT AGTTATCTAC ACCACGGGGAA GTCAGGCAAC TATGGATGAA CGAAATAGAC 1020  
 AGATGGCTGA GATACGTGCC TCACTGATTA ACCATTOGTA ACTGTCAGAC CAAGTTTACT 1080  
 CATATATACT TTAGATTGAT TTAATACCTTC ATTTTTAATT TAAAGGATC TAGGTGAAGA 1140  
 TCCCTTTTGA TAATCTCATG ACCAAATCC CTTAACGTGA GTTTTGTTC CACTGACCGT 1200  
 CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAACT 1260  
 GCTGCTTCCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTGCCG GATCAAGAGC 1320  
 TACCAACTCT TTTTCCGAA GTAATGGCT TCAGGAGAGC CGAGATAACCA AATACTGTCC 1380  
 TTCTACTGTA CGCGTAGTTA CGCCACCACT TCAAGAACTC TGTAGCAAGG CGTACATACC 1440  
 TCGCTCTGCT AATCTGTTA CGACTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG 1500  
 CGTGGACTC AAGACGATAG TTACCGATA AGGCAGACCG CGCGGGCTGA CGGGGGGTT 1560  
 CGTGCACACA CGCCAGCTG CGCGGAACGA CGTACGATAC CTACAGGCTG 1620  
 AGCATTGAGA AAGCGCCAGG CTTCCGGAAAG CGAGAAAGGC CGACAGGTAT CGGGTAAGGC 1680  
 CGACGGTCGG AACAGGAGAG CGCACCGAGGC ACCTTCAGG GGGAAACGCC TGGTATCTT 1740  
 ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGGCTCG ATTGTTGATGA TGCTCGTCAG 1800  
 CGGGGGCGAG CCTATGGAAA AACGCCAGCA ACCGGGGCTT TTTACGGTTTC CTGGCCTTTT 1860  
 CCTGGCCTTT TGCTCACATO TTCTTCCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA 1920

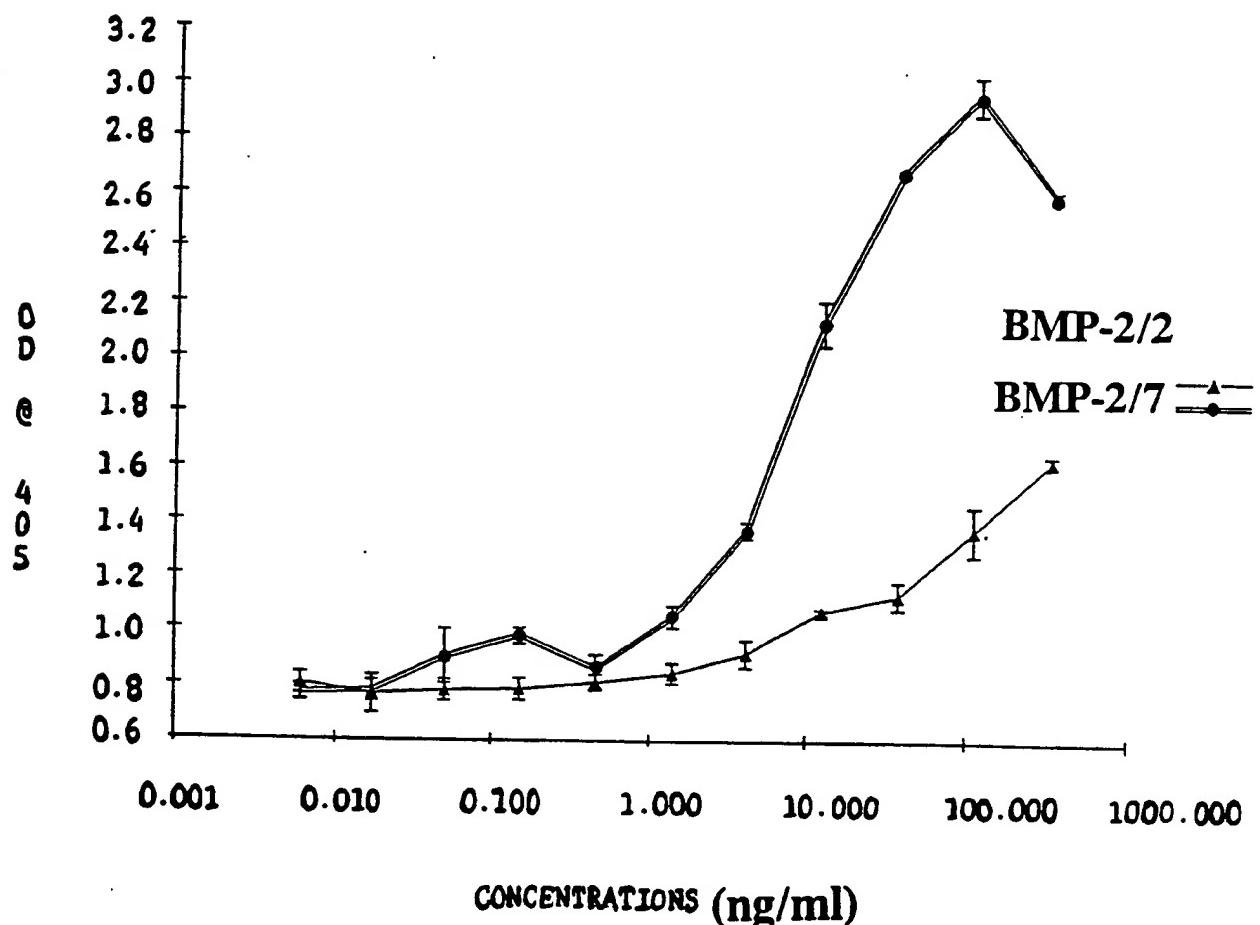
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## FIGURE 7 (cont'd)

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 CAGTGACCGA CGAACCGGAA GAGCGCCCAA TACGCCAACC GCCTCTCCCC CGCGGTTGGC 2040  
 CGATTCAATT ATGCAGAATT GATCTCTCAC CTACCAACA ATGCCCCCTT GCAAAAAATA 2100  
 AATTCAATA AAAAACATAAC AGATAACCAT CTGCGGTGAT AAATTATCTC TGGCGGTGTT 2160  
 GACATAATA CGACTGGCGG TGATACTGAG CACATCAGCA GGACGCAC TG ACCACCATGA 2220  
 AGCTGACGCT CTTAAAMATT AAACCCCTGA GAAAGGCAGC ATTCAAGCA GAAAGCTTTG 2280  
 GGGTGTGTA TACGAAACGA AGCATTGCC GTAAGTCCGA TTCCGGATTA GCTGCCAATG 2340  
 TCCCCATGCC GGGGGGTTT CGTTCAAGGAC TACAACTGCC ACACACCCACC AAGCTTAAC 2400  
 GACAGGAGAA TCCAGATGCA TGCACAAACA CGCCCCCGCG AACGTCGCC AGAGAAACAG 2460  
 GCTCAATGGA AACGAGCAA TCCCCCTGTT GTTGGGTTAA GCGAAACACC AGTCCGAAA 2520  
 GATTTTTTA ACTATAACG CTGATGCCAG CGTTTATGCC GAAAGACGTA AGCCCTTCCC 2580  
 GAGTAACAAA AAAACAAACAG CTTAAATAAC CGCGCTCTTA CACATTCCAG CCCTGAAAAA 2640  
 GGGCATCAA TTAAACCAAC CCTATGGTGT ATGCATTATAT TTGCAACAT TCAATGAAATT 2700  
 GTTATCTAG GAAATACCTA CATATGCCAG CTAAACATAA ACACGTAAA CGTCTGAAAT 2760  
 CTAGCTGTAA GACACACCCCT TTGTACGTGG ACTTCAGTOA CGTGGGGTGG AATGACTGGA 2820  
 TTGTGGCTCC CGCCGGGTAT CACGCCCTTT ACTGCCACGG AGAAATCCCT TTTCCCTCTGG 2880  
 CTGATCATCT GAACTCCACT AATCATGCCA TTGTTCAAGAC GTTGGTCAAC TCTGTTAACT 2940  
 CTAAGATTCC TAAGGCATGC TGTGTCCCGA CAGAACTCAG TGTATCTCG ATGCTGTACG 3000  
 TTGACCGAA TGAAAGGTT GTATTAAAGA ACTATCAGGA CAGGGTTGTC GAGGGTTGTC 3060  
 CCTGTCGCTA GTACAGCAA ATTAATACA TAAATATATA TATATATATA TATTTAGAA 3120  
 AAAAGAAAAA AATCTAGAGT CGACCTUCAG TAATCGTACA GGGTAGTACA AATAAAAAAG 3180  
 GCAOGTCAGA TGACCGTGCCT TTTTCTTGT GAGCAGTAAG CTTGGCACTG GCGCTCGTT 3240  
 TACAACGTG TGACTGGAA AACCCCTGGCG TTACCCCACT TAATCGCCTT GCAAGCACATC 3300  
 CCCCTTTCGG CAGCTGGCGT AATACCGAAG AGGGCCGAC CGATCGCCCT TCCCAACAGT 3360  
 TGCGCAAGCCT GAAATGGCGAA TGGCGCTGA TGGCGTATTT TCTCCTTACG CACCTGTGG 3420  
 GTATTTCAAA CGCGATATAT CGTGCACCTCT CAGTACAAATC TGTCTGATG CGCGCATAGTT 3480  
 AAGCCAGCCC CGACACCCCGC CAACACCCCG TGACCGACCC CGACGGGCTT GTCTGCTCCC 3540  
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 ACCGTCATCA CGAAACCCCG CGA 3623

**FIGURE 8****W-20 ALKALINE PHOSPHATASE: BMP-2 VS. BMP-2/7****SUBSTITUTE SHEET**

**FIGURE 9****EFFECTS OF BMP-2 AND BMP2/7 ON BGP SYNTHESIS BY W-20 CELLS****SUBSTITUTE SHEET**

**FIGURE 10****COMPARAISON OF *E.Coli* BMP-2 AND BMP-2/7:  
W-20-17 ALKALINE PHOSPHATASE****SUBSTITUTE SHEET**

## FIGURE 11A

10            20            30            40            50            60            70  
AGATCTTGTAA AACACCCGGG CCACACACGC CGCGACCTAC AGCTCTTCT CAGCGTTGGA GTGGAGAOGG

80            90            100          110          120          130          140  
CGCCCGCAGC GCGCTGOGOG GGTGAGGTCC CGCGAGCTGC TGGGAAAGAG CGCACCTGTC AGGCTGOGCT

150          160          170          180          190          200          210  
GGGTCAAGCAGC AGCAAGTGGG GCTGGGOGCT ATCTCGCTGC ACGGGCGC GTGGGGGCT CGTGCGCC

220          230          240          250          260          270          280  
TOGCCCGCAGC TGGTTTGGAG TTCAACOCTC GGCTCGCGCG CGCGCTCTT GOGCGTTGG AGTGTCCCGC

290          300          310          320 (1)        335  
AGCGAOGCG AGGAGCGAOG CGCGCGCGGG GTACCTAGCC ATG GCT GGG GCG AGC AGG CTG CTC  
MET Ala Gly Ala Ser Arg Leu Leu

350          365          380          395  
TTT CTG TGG CTG GCC TGC TTC TGC GTG AGC CTG CGC CAG GGA GAG AGA CGG AAG CCA  
Phe Leu Trp Leu Gly Cys Phe Cys Val Ser Leu Ala Gln Gly Glu Arg Pro Lys Pro

410          425          440          455  
CCT TTC CGG GAG CTC CGC AAA GCT GTG CCA GGT GAC CGC ACG GCA GGT GGT GGC CGG  
Pro Phe Pro Glu Leu Arg Lys Ala Val Pro Gly Asp Arg Thr Ala Gly Gly Pro

470          485          500          515  
GAC TCC GAG CTG CAG CGC CAA GAC AAG GTC TCT GAA CAC ATG CTG CGG CTC TAT GAC  
Asp Ser Glu Leu Gln Pro Gln Asp Lys Val Ser Glu His MET Leu Arg Leu Tyr Asp

530          545          560  
AGG TAC AGC ACG GTC CAG CGC CGG ACA CGG GGC TCC CTG GAG GGA CGG TCG CAG  
Arg Tyr Ser Thr Val Gln Ala Ala Arg Thr Pro Gly Ser Leu Glu Gly Ser Gln

575          590          605          620  
CCC TGG CGC CCT CGG CTC CTG CGC GAA GGC AAC ACG GTT CGC AGC TTT CGG CGG GCA  
Pro Trp Arg Pro Arg Leu Leu Arg Glu Gly Asn Thr Val Arg Ser Phe Arg Ala Ala

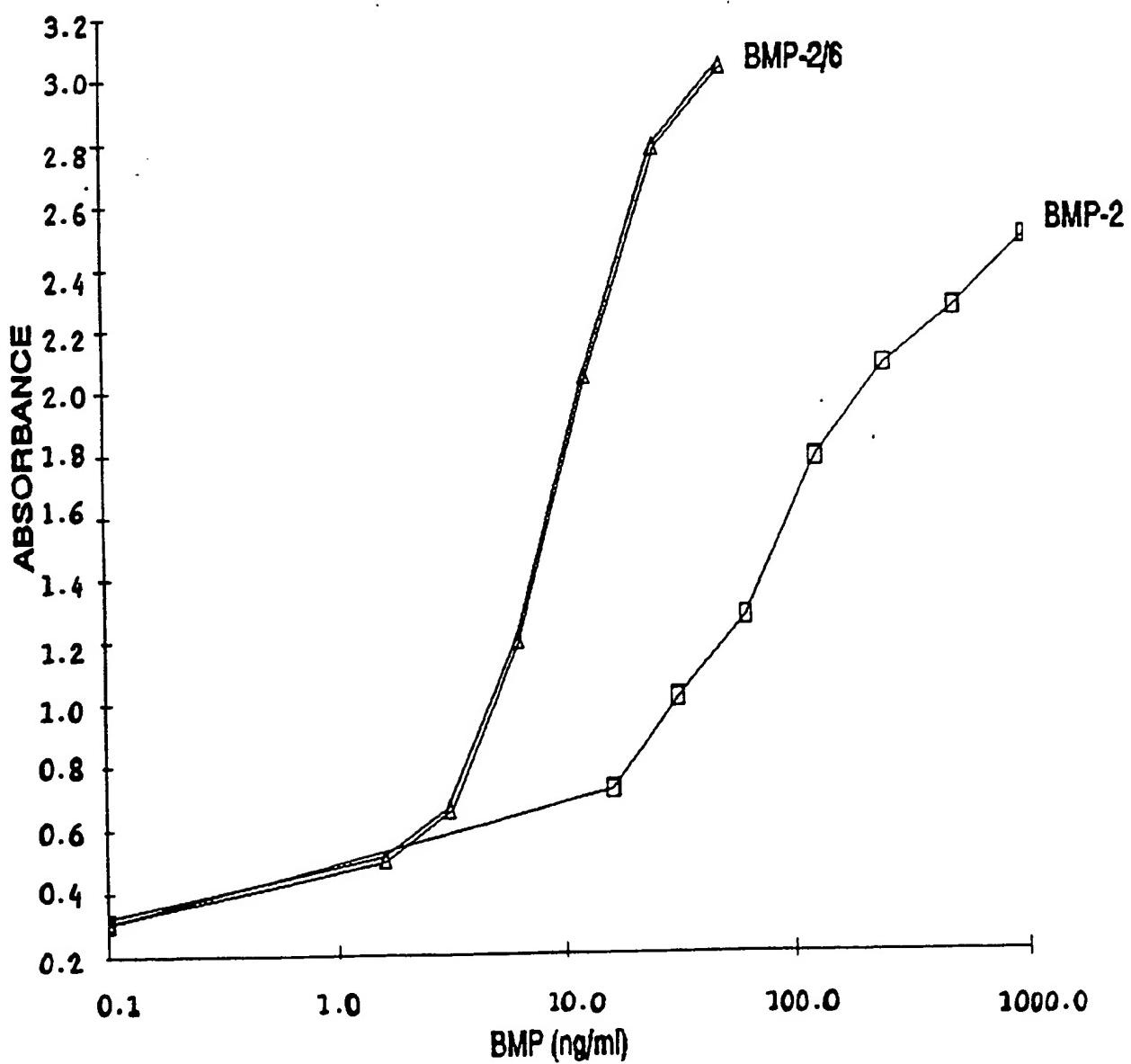
635          650          665          680  
GCA GCA GAA ACT CTT GAA AGA AAA GGA CTG TAT ATC TTC AAT CTG ACA TCG CTA ACC  
Ala Ala Glu Thr Leu Glu Arg Lys Gly Leu Tyr Ile Phe Asn Leu Thr Ser Leu Thr

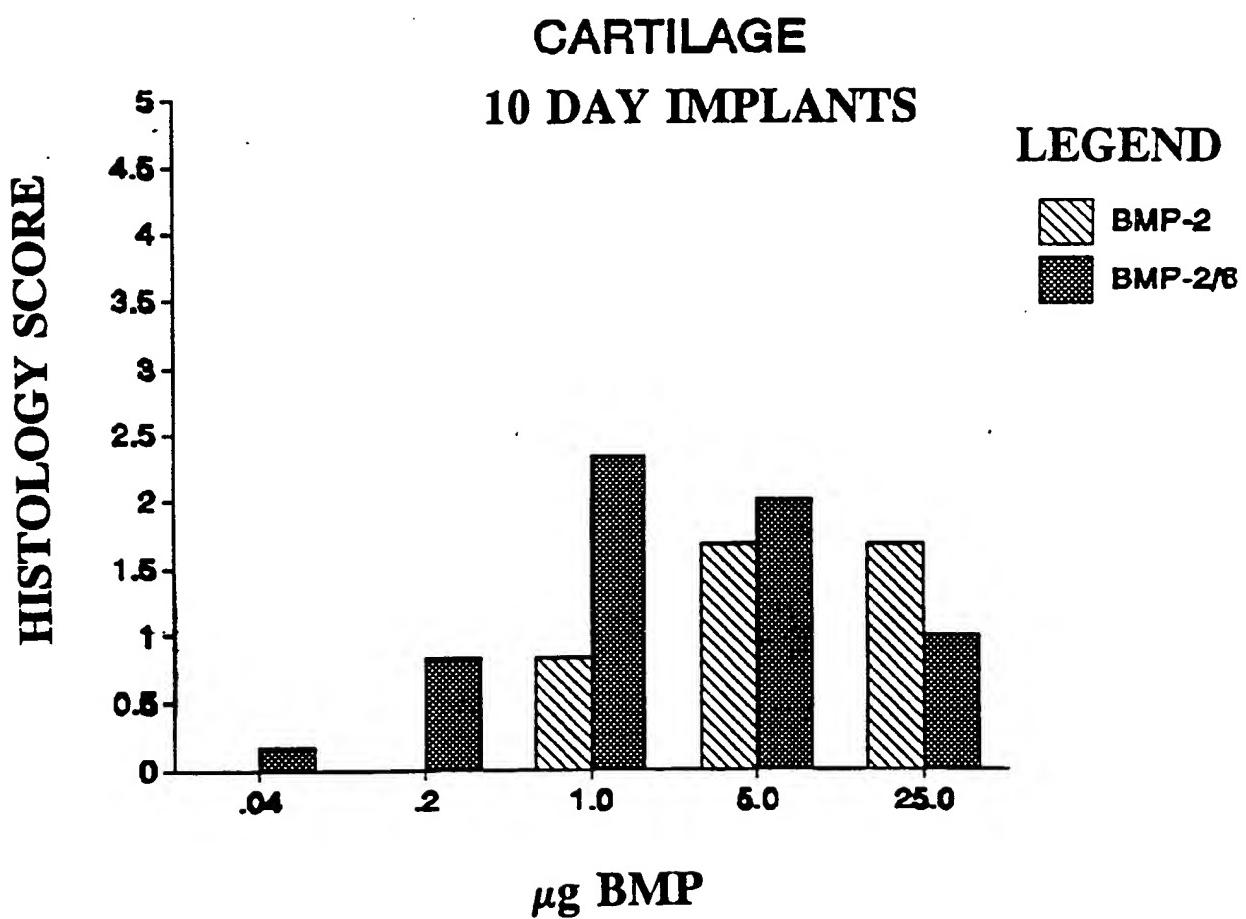
695          710          725          740  
AAG TCT GAA AAC ATT TTG TCT GCC ACA CTG TAT TTC TGT ATT GGA GAG CTA CGA AAC  
Lys Ser Glu Asn Ile Leu Ser Ala Thr Leu Tyr Phe Cys Ile Gly Glu Leu Gly Asn

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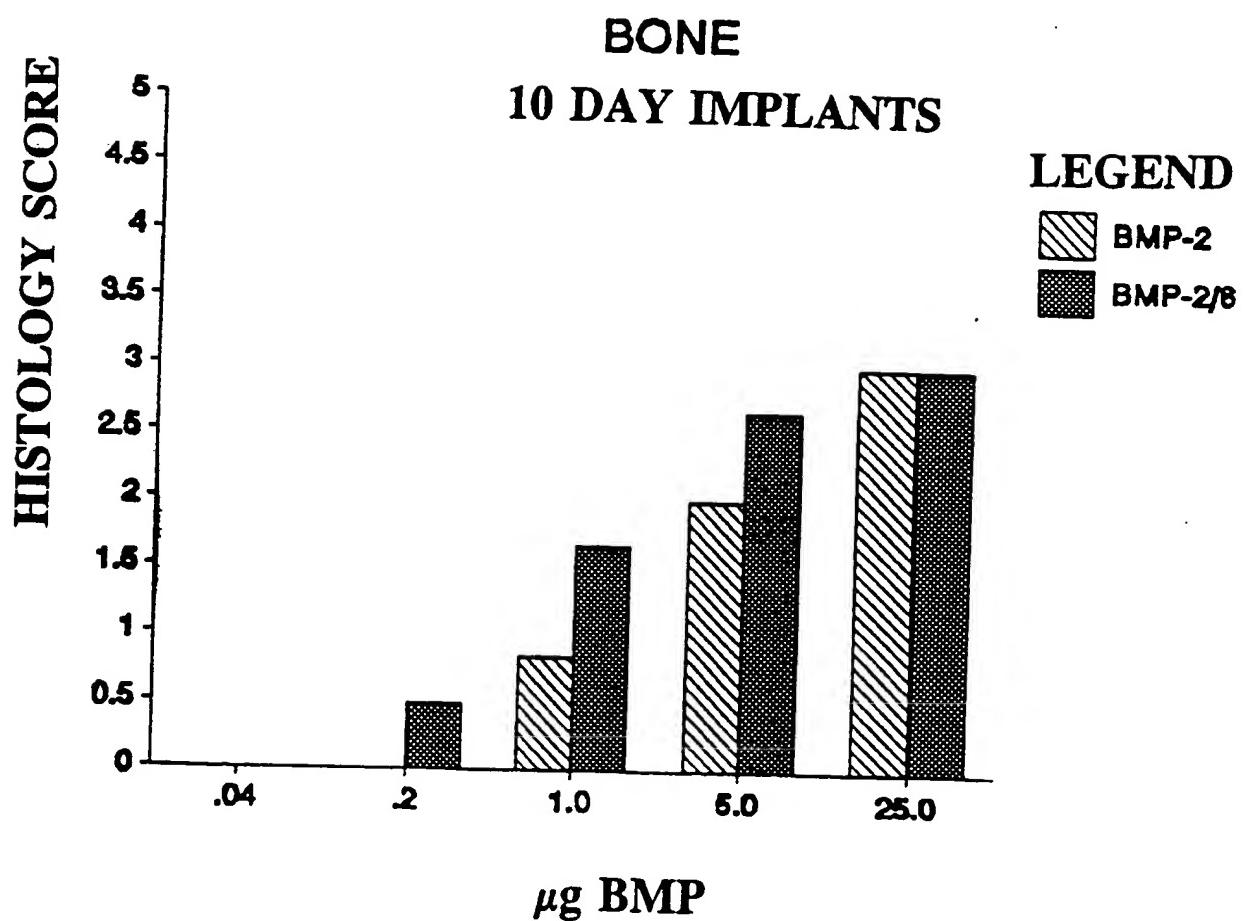
## FIGURE 11C

1430                  1445 (377)                  1460                  1475  
 TGC GGC AGG AGA TAC CTC AAG GTA GAC TTT GCA GAT ATT GGC TGG AGT GAA TGG ATT  
 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser Glu Trp Ile  
  
 1490                  1505                  1520                  1535  
 ATC TCC CCC AAG TCC TTT GAT GGC TAT TAT TGC TCT GGA GCA TGC CAG TTC CCC ATG  
 Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro MET  
  
 1550                  1565                  1580                  1595  
 CCA AAG TCT TTG AAG CCA TCA AAT CAT GCT ACC ATC CAG AGT ATA GTG AGA GCT GTG  
 Pro Lys Ser Leu Lys Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val  
  
 1610                  1625                  1640                  1655  
 GGG GTC GTT CCT GGG ATT CCT GAG CCT TGC TGT GTA CCA GAA AAG ATG TCC TCA CTC  
 Gly Val Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Glu Lys MET Ser Ser Leu  
  
 1670                  1685                  1700  
 AGT ATT TTA TTC TTT GAT GAA AAT AAG AAT GTA GTG CTT AAA GTA TAC CCT AAC ATG  
 Ser Ile Leu Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn MET  
  
 1715                  1730 (472)                  1746                  1756                  1766                  1776  
 ACA GTA GAG TCT TGC GCT TGC AGA TAACCTGGCA AAGAACTCAT TTGAATGCTT AATTCAATCT  
 Thr Val Glu Ser Cys Ala Cys Arg  
  
 1786  
 CTAGAGTCGA CGGAATTG

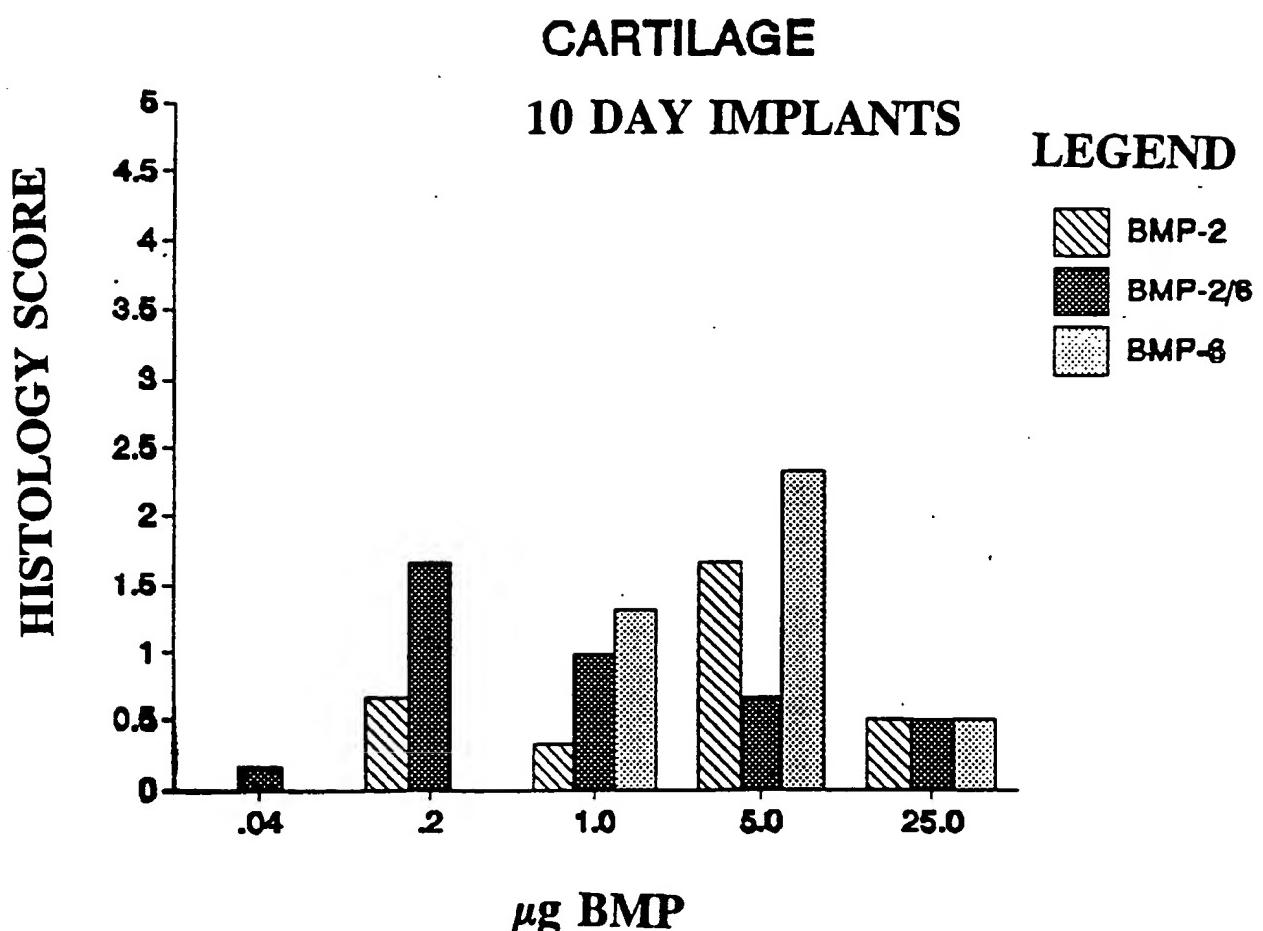
**Figure 12****W-20 ALKALINE PHOSPHATASE: CHO BMP-2/6 vs. CHO BMP-2****SUBSTITUTE SHEET**

**FIGURE 13A**

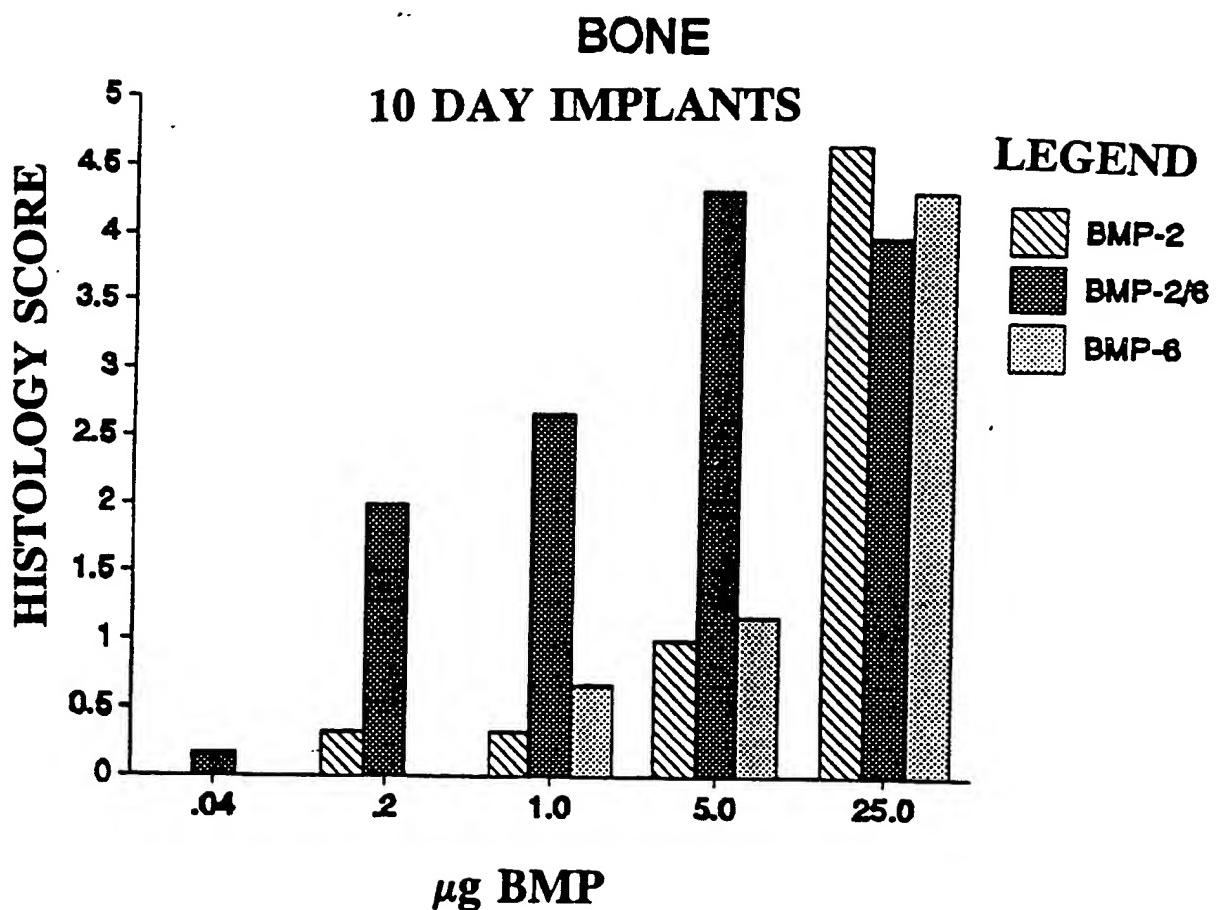
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**FIGURE 13B**

**SUBSTITUTE SHEET**

**FIGURE 14A**

**SUBSTITUTE SHEET**

**FIGURE 14B**

SUBSTITUTE SHEET

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.C1. 5 C12N15/12;	C12P21/02;	A61K37/02;	C12N5/12
C07K15/06			

**II. FIELDS SEARCHED**Minimum Documentation Searched<sup>7</sup>

Classification System	Classification Symbols		
Int.C1. 5	C07K ;	C12N ;	A61K ; C12P

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>**III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>**

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	WO,A,9 003 733 (INTERNATIONAL GENETIC ENGINEERING, INC.) 19 April 1990 see page 16, line 7 - page 17, line 28 see page 18, line 22 - line 34 see page 51, line 32 - page 52, line 10; figure 12 see page 62 - page 63; claim 35 ----	1,4, 7-14,16, 23-26
Y	WO,A,9 011 366 (GENETICS INSTITUTE, INC.) 4 October 1990 cited in the application see page 22, line 20 - line 27 see page 43, line 17 - line 30 ----	13-17, 33,35
Y	-----	13-16,33

<sup>10</sup> Special categories of cited documents :<sup>10</sup>

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Date of the Actual Completion of the International Search

04 FEBRUARY 1993

Date of Mailing of this International Search Report

26.02.93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

ANDRES S.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT		(CONTINUED FROM THE SECOND SHEET)	
Category <sup>a</sup>	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.	
X	WO,A,8 910 409 (GENETICS INSTITUTE, INC.) 2 November 1989 cited in the application see page 7, line 13 - line 15 see page 8, line 20 - line 29 ---	1,4, 7-12,23, 25-26	
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, March 1990, WASHINGTON US pages 2220 - 2224 WANG, E.A. ET AL. 'Recombinant human bone morphogenetic protein induces bone formation' cited in the application see figure 1C ---	34,36	
Y		35	
X	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 265, no. 22, 5 August 1990, BALTIMORE, MD US pages 13198 - 13205 SAMPATH, T.K. ET AL. 'Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily' see the whole document ---	34,36	
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, no. 24, December 1990, WASHINGTON US pages 9843 - 9847 CELESTE, A.J. ET AL. 'Identification of transforming growth factor-beta family members present in bone-inductive protein purified from bovine bone' see page 9846, left column, line 13 - right column, line 7 see page 9847, left column, paragraph 2-3 ---	34,36	
A		13,16	
A	WO,A,8 909 787 (CREATIVE BIOMOLECULES, INC.) 19 October 1989 see page 6, line 22 - line 24 see page 56, paragraphs E5 & E6 ---		
P,Y	WO,A,9 118 098 (GENETICS INSTITUTE, INC.) 28 November 1991 cited in the application see page 12, line 31 - page 13, line 7 ---	17	
		-/-	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category <sup>a</sup>	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	JOURNAL OF CELLULAR BIOCHEMISTRY Supplement 16F, 1992, page 76, abstract WO26; WOZNEY, J.M. ET AL.: 'Regulation of chondrogenesis and osteogenesis by the BMP proteins' see abstract & Keystone Symposium on growth and differentiation factors in vertebrate development; Keystone, Colorado, USA April 3-16, 1992 -----	1

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9209430  
SA 66918

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		EP-A-	0429570	05-06-91
		JP-T-	3505098	07-11-91
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		AU-B-	628050	10-09-92
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		AU-A-	3530589	03-11-89
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		EP-A-	0362367	11-04-90
		JP-T-	3500655	14-02-91
		JP-T-	3502579	13-06-91
		WO-A-	8909788	19-10-89
		US-A-	5108753	28-04-92
		AU-B-	627850	03-09-92
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		EP-A-	0411105	06-02-91
		JP-T-	3504736	17-10-91
		WO-A-	9010018	07-09-90
		US-A-	4975526	04-12-90
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		US-A-	5162114	10-11-92
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ON INTERNATIONAL PATENT APPLICATION NO.**

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9118098	28-11-91	None	

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